ARSENIC BIOMONITORING IN RURAL NOVA SCOTIA, CANADA

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We, the undersigned, certify that **David J. McIver**, candidate for the degree of Doctor of Philosophy has presented his thesis with the following title: "Arsenic Biomonitoring in Rural Nova Scotia, Canada" and that the thesis is acceptable in form and content, and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on January 8, 2013.

Examiners

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_____ Dr. Jeffery Davidson (Chair)

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Abstract

The objectives of this thesis were: 1) to determine which factors and biological samples were associated with the estimated amount of total arsenic (As) consumed from water and food; 2) to determine which factors were associated with the concentration of total and speciated As ([As]) in urine; 3) to determine which factors were associated with the total [As] in nails and hair; and 4) to evaluate a novel method of quantifying As in human nails via x-ray fluorescence (XRF).

In 2010, a questionnaire was administered to 179 people from two locations in Nova Scotia, Canada. The questionnaire collected information on participants' eating and drinking habits, and key social and demographic information such as: cigarette and alcohol consumption, length of time in home, household income, etc. Water and biological samples, collected from each participant, were analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to quantify the total concentration of As ([As]) and selenium (Se) in each. Total water [As] ranged from <0.02 – 309.2 μg/L. Total [As] of urine (adjusted by specific gravity, SG), fingernails, toenails, and hair ranged from <0.71 – 661.1 μg/L, from <0.07 – 2.3 μg/g, from <0.15 – 4.1 μg/g, and from <0.15 – 13.2 μg/g, respectively.

Total urinary [As] was associated with estimated total amounts of As consumed from water and food in the last 3 days. Total fingernail [As] and water pH were also associated with the amount of As consumed from water, and income was associated with food As consumption. The depth of the well used to draw

drinking water was associated with water As consumption and total As consumption, especially between the depths of 22 – 55 m.

High Performance Liquid Chromatography was used to measure arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA(V)), dimethylarsinic acid (DMA(V)), arsenobetaine (AsB), and total urinary arsenic metabolites minus arsenobetaine (TMA), in urine. Total water [As] was a strong positive predictor of all urinary As metabolite concentrations, with the exception of AsB. Consumption of seafood was positively associated with urinary concentrations of total As and AsB, but also As(III), DMA(V), and TMA, suggesting that seafood-derived As may be metabolized in the body to other As metabolites. Both total [As] and [AsB] in urine were found to be slightly higher in older participants. Participants who reported recent consumption of chicken also had slightly increased concentrations of urinary As(V). Finally, a strong positive relationship was found between water pH and MMA(V), DMA(V), and TMA concentrations in urine.

Multiple linear and logistic regression analyses found that water [As] and sample [Se] were predictors of [As] in hair and nails. This finding supports previous research which has found a high degree of interaction between As and Se in humans, which is important to consider when performing an As biomonitoring study.

Evaluation of the XRF found the concordance correlation coefficient and kappa value were highest for fingernail samples of at least 20 mg in mass, but overall, XRF did not compare well to ICP-MS. Using a clinically relevant

concentration of 1.0 μg/g on ICP-MS as a cut-off for indicating test positive and test negative samples, the XRF, also using a cut-off of 1.0 μg/g, was found to have 100% sensitivity (95% CI: 48 – 100%) and negative predictive value (95% CI: 63 – 100%), however its specificity and positive predictive values were low, at 29% (95% CI: 17 – 43%) and 19% (95% CI: 8 – 33%), respectively.

Further research involving urine as a biological marker of exposure to As should take specific urinary As metabolites into consideration and pay special attention to seafood consumption, which can strongly affect urinary [As], and can potentially lead to an increase in toxic inorganic As metabolites. Studies of As in hair or nails should consider the total and relative concentrations of Se in respective samples. More work is needed before the XRF can be considered a suitable replacement for ICP-MS.

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List of Abbreviations

As	Arsenic
As(III)	Arsenite
As(V)	Arsenate
AsB	Arsenobetaine
ATSDR	Agency for Toxic Substances and Disease Registry
CCC	Concordance Correlation Coefficient
CI	Confidence Interval
Coef	Coefficient
DMA(III)	Dimethylarsinous Acid
DMA(V)	Dimethylarsinic Acid
DOE	Department of Environment
EPA	Environmental Protection Agency
HPLC	High Performance Liquid Chromatography
iAs	Inorganic Arsenic
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
keV	Kilo-electron volts
m	Meter
MAC	Maximum Allowable Concentration
MCL	Maximum Contaminant Limit
MDL	Minimum Detection Limit
mg/kg	Milligram per Kilogram
ml	Millilitre
MMA(III)	Monomethylarsonous Acid
MMA(V)	Monomethylarsonic Acid
ng/g	Nanogram per Gram
NPV	Negative Predictive Value
NS	Nova Scotia
OR	Odds Ratio
Pb	Lead
PPV	Positive Predictive Value
Se	Selenium
SG	Specific Gravity
Sn	Sensitivity
Sp	Specificity
TMA	Total Arsenic Minus Arsenobetaine
μg	Microgram
μg/g	Microgram per Gram
μg/L	Microgram per Litre
XRF	X-ray Fluorescence
WHO	World Health Organization

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1 Introduction and Literature Review

1.1 Global Distribution of Arsenic

Throughout the world, arsenic (As) is commonly found in the environment, occurring naturally in small amounts in air, soil and water (World Health Organization, 2008) (WHO). However, in some areas the [As] in soil, groundwater and air can reach dangerous levels through both natural and anthropogenic pathways. In the natural environment, As is rarely found in its pure form and is most often found as a complex with other elements. Inorganic As is found primarily as arsenate (AsO_4^{3-}) in oxidizing (aerobic) soils, and as arsenite (AsO_3^{3-}) under reducing (anaerobic) conditions (Canadian Council of Ministers of the Environment, 1997). As can be found in high concentrations in areas rich in volcanic rock, marine sedimentary rock, and coal deposits (Wang & Milligan, 2006), and it is the subsequent leaching out of As from these soils and bedrock into the underground water reservoirs which results in the high [As] in drinking water (Walsh & Keeney, 1975). Anthropogenically derived As is most often a result of industrial activities that either use As as a production material or produce As as a waste product. Examples of these industrial activities which lead to high water [As] include: non-ferrous metal mining and smelting, wood preservation, coal combustion, herbicide and pesticide production and use, semiconductor electronic devices production, and manufacturing and waste incineration (Woolson, 1977). Industrial activities such as coal or fossil fuel combustion have been shown to increase the amount of As in the air in surrounding areas, leading to increased [As]

in urine and hair (Bencko, Rames, Fabiánová, Pesek, & Jakubis, 2009; Shraim et al., 2003). Neither of the two locations selected for the present study, Hubbards and Fall River, are situated directly near any industrial activities known to increase the amount of As in the air, making this a possible, but likely negligible, mode of potential As exposure to the populations in these areas.

Through improper management and disposal, As can be deposited onto surface soil and enter into surface water and leach deeper into underground waterways, which can distribute the contaminant over a large area. A "normal" [As] in groundwater is between 1 – 10 μg/L, whereas in some areas of the world, concentrations of up to 3200 μg/L have been reported in scientific literature (Biswas et al., 2010). Countries that have areas of particular concern for As poisoning in groundwater include: India (especially West Bengal), Bangladesh, China, Thailand, and Chile, among others. An example of a problem area which is perhaps more relevant to Canada is Nevada, USA, where an average of 1,312 μg/L of As was recorded in the main water source for the exposed group in a cross sectional study (Warner et al., 1994). Most areas of the world are not at risk of high As exposure through natural groundwater, however there are often isolated high-risk areas found within low-risk areas. Within these high-risk areas, populations that are most at risk include those with little or no access to water quality testing or medical staff able to recognize the signs of chronic As poisoning, and those with little money to implement any type of filtration systems.

1.2 Arsenic in Nova Scotia

Many areas of Nova Scotia, Canada, are currently known to have [As] in groundwater that surpasses the international standard of 10 μg/L, as set by the WHO. Though much of the affected areas are high in As due to geological anomalies, there are several areas of concern that have been influenced by human activities. For example, large areas of soil and groundwater surrounding the retired Montague gold mines are contaminated with As (Meunier et al., 2010). These contaminated areas are sources for As to leach into underground waterways, which can allow the dispersal of As over large areas and into private wells used for drinking water (World Health Organization, 2008). In 1997, the Canadian Council of Ministers of the Environment reported that out of 433 soil samples taken from areas near retired gold mines in Nova Scotia, the mean [As] in the samples was 10,000 mg/kg (range of 9.0 mg/kg – 310,000 mg/kg). To put this into context, 99% of the samples were above the regulatory limit of 12 mg/kg (Canadian Council of Ministers of the Environment, 1997). Samples taken near the Montague Mines area, which is adjacent to Fall River, NS, ranged from 320 mg/kg to 62,000 mg/kg of As, with a mean of 22,387 mg/kg of As. All of the samples taken were above the recommended [As] in soil, ranging from 27 times to over 5,000 times the recommended limit. (Meliker, Goovaerts, Jacques, et al., 2010)

1.3 Arsenic Toxicokinetics

Arsenic can exist in a number of organic and inorganic states, known as species (see Figure 1.1). The inorganic states predominate in groundwater, existing mainly as arsenate [As(V)]. In what has been largely recognized as a chain of

metabolic detoxification, arsenate (As(V)) is initially reduced by enzymes (arsenate reductase) in the liver to form arsenite [(As(III)]. This is followed by the methylation of arsenite to monomethylarsonic acid (MMA(V)), which is reduced to monomethylarsonous acid (MMA(III)). MMA(III) is then methylated to dimethylarsinic acid (DMA(V)) that is reduced, in turn, to dimethylarsinous acid (DMA(III)) (Vahter, 2002). Within the last decade, researchers such as Kitchin et al. (2001), have questioned the proposed "detoxification" of arsenate to arsenite, suggesting that the latter may perhaps be even more toxic than the former. As well, while inorganic As may have more serious acute effects than the methylated forms (Vahter, 2000), these methylated species are possible cancer promoters and are causes for concern in the long-term (Kitchin, 2001). Research on both organic and inorganic forms of As is currently ongoing.

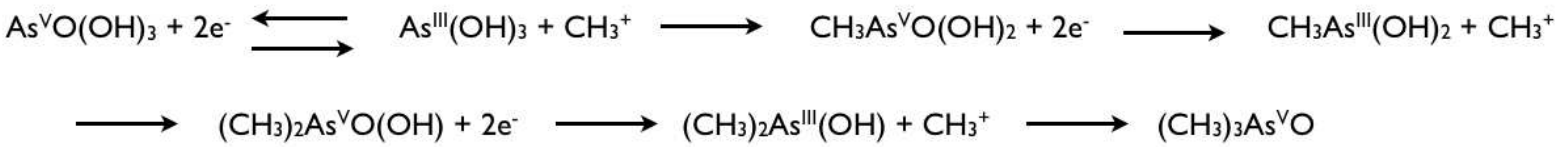

Figure 1.1 Metabolic pathway of arsenic in humans

Arsenobetaine is an organic form of As found primarily in seafood. While traditionally held that As ingested from seafood is excreted in the urine largely unchanged, and AsB has been found to do just that, recent research has shown that some compounds, such as arsenolipids and arsenosugars, are metabolized to DMA(V) and over a dozen other metabolites (Francesconi, Tanggaar, McKenzie, et al., 2002; Schmeisser et al., 2006). Many of the AsB metabolites have not been characterized and their toxicokinetic profiles are unknown.

When considering acute As toxicity, ingestion of as little as 2 mg of inorganic As trioxide has been documented as sufficient to induce vomiting, diarrhoea, severe abdominal cramps and death in adults (Levin-Scherz, Patrick, Weber, et al., 1987). However, the accepted normal range of an acutely lethal dose of inorganic As is between 100 – 300 mg, with death normally occurring within 24 hours to 4 days post-exposure (Ratnaike, 2003).

Chronic exposure to elevated levels of As can lead to many deleterious health effects. Many different organs, including skin, kidney, liver and lungs, are more likely to develop cancers when an individual is exposed to As (Tsai, Wang & Ko, 1999). Exposed individuals are also at an increased risk of developing hyper- or hypopigmented regions on their hands and feet, which are often a precursor to basal and squamous cell skin cancers (Ahsan et al., 2006; Chiou et al., 2001; Kapaj, Peterson, Liber, et al., 2006; Yu, Liao & Chai, 2006). Peripheral neuropathy has also been reported (Chen et al., 2009), as well as decreased cognitive achievement in children exposed to As (Asadullah, 2008; Chen et al., 2009). Studies have also reported negative As-related health outcomes related to sexual reproduction,

including: decreased infant birth weight, increased infant mortality, increased anemia during pregnancy, and decreased semen quality (Hopenhayn et al., 2003; Hopenhayn, Bush, Bingcang, et al., 2006; Hopenhayn-Rich et al., 2000; Xu et al., 2012).

As a genotoxic substance, As interferes with the normal processes of DNA maintenance and replication. Because As has similar chemical properties to phosphorus, it can be used in place of phosphorus in some reactions. Additionally, As can interfere with signal transduction pathways for the activation of enzymes responsible for repairing damaged DNA. For example, several enzymes that help in the repair of damaged sections of DNA, such as poly(ADP-ribose) polymerase (Yager & Wiencke, 1997), are inhibited by free phosphorus and in cases when there is a high level of inorganic As, it is able to bind to these enzymes which reduces or inhibits their ability to repair the DNA, potentially leading to cancer development (Basu, Mahata, Gupta, et al., 2001). As well, underlying deficiencies in a person's DNA repair efficiency (i.e., suppressed DNA repair gene expression) have also been shown to play a role in the extent of damage caused by As; a lack of ability to repair broken DNA further compounds the damaging effects of (Ahsan et al., 2003; Andrew, Karagas & Hamilton, 2003; Banerjee et al., 2008).

There exists a complex relationship between As and selenium (Se) in terms of their metabolism and interactions in humans. As an essential trace nutrient, both low and high levels of Se in people can be detrimental to health (Brown & Arthur, 2001). It has been shown that the negative health effects of over or underexposure to Se can be altered by consumption of As, and that, similarly, the negative health

effects of As exposure can be ameliorated by supplementation of Se in the diet (Levander, 1977; Zwolak & Zaporowska, 2011). Studies have shown that in As affected areas, groups of individuals who receive selenium supplements in their diet have less As in blood and hair (Linsheng Yang, Wang, Hou, Peterson, & Williams, 2002), and it is suspected that this effect could be due to an arsenic-selenium conjugate molecule, which facilitates the mutual excretion of these two elements (Gailer, 2009; Korbas, Percy, Gailer, & George, 2008). Due to these metabolic interactions between As and Se, the amount of Se in water and biological samples was also of interest in this thesis.

1.4 Quantification of Arsenic Consumption

1.4.1 Quantifying Total Arsenic Consumption

It is possible to estimate the amount of As an individual is exposed to by measuring several key variables. First, and most importantly, the measurement of a person's primary water source for the presence of As is needed. A second requirement is an estimation of the quantity of water a person is drinking. Logically, someone whose water [As] is 500 μg/L is still technically unexposed if they never drink this water, whereas someone whose water [As] is 13 μg/L is more exposed if they drink large amounts of water. Third, gathering information on eating habits is also important. It is known that certain foods, especially seafood, can contain high levels of As (Schoof et al., 1999; Wrench, Fowler & Ünlü, 1979). From this information, a quantification of total daily As intake can be estimated, with the approximate formula of:

$$Arsenic\ Consumed = \quad water\ \ As\ \ \frac{ug}{L} \times water\ consumption\ \ L \quad +$$

$$food\ \ As\ \ \frac{ug}{g} \times food\ consumption\ \ g$$

1.4.2 Quantifying Arsenic Consumption from Food

To determine a person's total exposure to As, the amount of As consumed in food must be taken into account. When conducting a field study, it is costly and impractical to collect samples of each type of food a participant has eaten in the last 48-72 hours. As such, researchers must come up with a likely amount of As consumed by conducting a detailed questionnaire on eating habits, and combining these data with published reference values for levels of As in common foods.

Several publications provide an overview for the [As] in many different types of foods (Cascio et al., 2010; Dabeka, McKenzie & Lacroix, 1987; Dabeka et al., 1993; Schoof & Yager, 2007; Schoof et al., 1999). In addition, there have been many studies that have used published information on [As] in food to complete an epidemiological study of an As exposed population (Muñoz et al., 2005; Xue, Zartarian, Wang, et al., 2010). Although general trends in amount of As, and distribution of As species (As(III), As(V), MMA, DMA, organic) are similar across reports, concentrations of As in individual types of foods have some variability. Seafood (finfish and shellfish) are often recorded to have elevated total [As], with reported data ranging from 160 ng/g to 2,360 ng/g (Schoof et al., 1999). The dominant form of As in seafood samples is organic As, while inorganic As accounts for only a very small portion of the total amount of As in seafood, and is often immeasurable.

Rice tends to contain an elevated concentration of total As as well. Once again, it is organic As that is often found in elevated concentrations in rice, but inorganic As is found in higher concentrations in rice than any other tested foods. With [As] upwards of 73 μg/g (uncooked), rice presents a significant portion of consumed As for people whose diet is high in rice (Schoof et al., 1999).

Knowledge of consumption of these foods is important in order to account for amounts of total As found in different biological samples. By taking consumption practices for these and other food sources such as foods boiled in As containing water into consideration, a more accurate picture of As consumption is provided.

1.5 Tests for Detection of Arsenic

1.5.1 Detection of Arsenic in Water – Total and Speciated Analysis

The most used and most reliable method of determining the amount of As in a sample of water, and indeed of determining most elements in aqueous samples, is by way of inductively coupled plasma mass spectrometry (ICP-MS) (United States Environmental Protection Agency, 1994). In brief, the method entails ionizing elements in a sample by use of inductively coupled plasma. Once ionization is complete, the mass spectrometer separates and measures the elements based on mass-to-charge ratios, and the spectrometer receives a signal based on the concentration of ions produced from the sample. A larger signal corresponds to a high concentration of a particular element in a sample. ICP-MS analysis is often preferred to other analytical methods such as inductively coupled plasma atomic

emission spectrometer (ICP-AES) or an atomic absorption spectrometry technique (AAS) because of its ability to detect extremely small concentrations, down to the parts per trillion in some cases, and the wide range of accepted sample types. Low detection limits are acquired through time consuming and fine-tuned quality control procedures, which are required to be performed after approximately 10 analyses, to ensure calibrated results. An ICP-MS machine is a large piece of equipment that requires a dedicated laboratory space, and is costly to run. Therefore, their use is largely confined to well-funded laboratories.

In order to detect different As species in an aqueous sample, ICP-MS must be used in unison with High-Performance Liquid Chromatography (HPLC) (Komorowicz & Baralkiewicz, 2011). When used together, often abbreviated as HPLC-ICP-MS, it is possible to quantify total [As] as well as individual As species concentration. In this arrangement, HPLC is responsible for dividing the As in the sample into its constituent species. After this is complete, the sample is then processed by ICP-MS, which is now able to detect all As species and determine their concentration in the normal operating manner.

While an excellent technique for modern, well-equipped laboratories, ICP-MS and HPLC machines are large and bulky, making their using in field studies in remote areas quite impractical. In addition, rigorous quality control measures must be taken periodically through the sample analysis which adds to cost and time commitments. Typically, to analyze a single sample for a single element, the total cost of reagents and preparation materials is approximately $10 CAD, plus technician costs. Additionally, a standard ICP-MS machine is approximately

$250,000 CAD. A new technique for analyzing water and biological samples in a field environment, at a low cost, would be highly beneficial in areas of the world where conventional chemical analysis is not a viable option.

1.5.2 Detection of Arsenic in Human Biological Samples – Total and Speciated Analysis

Similar to a water sample, the most common method for detecting As in human tissues is ICP-MS. Both liquid (urine, blood) and solid (hair, nails) biological samples can be tested via ICP-MS. Depending on the sample being tested, various concentrations of As can be expected, based on the amount of As being consumed. Inorganic As is readily metabolized and quickly removed from the body. The majority of ingested As leaves the body through urine, but because of its strong affinity for keratin (due to the high sulfur content), a small portion is also incorporated into nails and hair.

It is possible to determine the amount of As consumption over different time periods by assessing individual tissues for the presence of As. Similar to a water sample, both total [As] and As species concentrations can be estimated from a urine sample using ICP-MS. Due to the step-wise conversion of inorganic acid to MMA(V) and then to DMA(V), most often the largest component of As in a urine sample is DMA(V) followed by MMA(V) with inorganic arsenic (iAs) concentrations being lower than both organic forms. In contrast, ingested organic As remains at a relatively stable level in the body and is eliminated largely unaltered (Mandal,

Ogra, Anzai, et al., 2004). As with water, urine can be analyzed for separate As species by using the HPLC-ICP-MS method.

To assess As exposure over a larger time scale, fingernails, toenails and hair can be used (Gault et al., 2008). As toenails grow more slowly than fingernails, they can provide a better understanding of As exposure over a longer period of time than fingernails. While hair grows more quickly than fingernails or toenails, which would imply a shorter time-frame of exposure assessment, people tend to have hair much longer than fingernails and toenails, and this presents an opportunity to assess exposure over an even longer time period than with nails. Exposure to As can be determined from fingernails and toenails for approximately 4-6 weeks prior, whereas hair, depending on length, can be an indicator of exposure for months or years. When using nails or hair to estimate As exposure, attention is required to ensure external sources of As are considered. When involved in activities that expose hair or nails to water, As in water can be absorbed into the hair or nails and would therefore lead to an overestimation of the true exposure concentration. In addition, it has been suggested that As found in human sweat may be a potential source of contamination of hair, because hair can absorb some of the As present in sweat, thereby leading to possible overestimation of [As] in hair (Hindmarsh, 2002).

The detection of As in blood samples is also possible, but it is not often used as a biomarker in environmental studies for several reasons. Arsenic in blood has a half-life of about 1-hour and is therefore cleared from the circulatory system very quickly (National Research Council, 2001). For this reason, blood sample analysis is

only appropriate for the investigation of a very recent exposure, such as accidental exposure or poisoning. Moreover, the [As] found in a blood sample are much lower than those found in a urine sample, making reliable analytical measurement more difficult to obtain. Finally, blood sample collection is more invasive than a urine sample, and thus is less likely to be accepted by research volunteers.

Although the analysis of biological samples by ICP-MS is very accurate and reliable, the areas most affected by high [As] in groundwater are most often distant from such equipment. Therefore, the populations who need the laboratory testing most urgently are also those most likely to not have access to it. To rectify this issue, a newly developed methods of As detection in human biological samples should be evaluated. A new method should be easily transportable, allowing it to reach populations in remote areas. As well, it should be substantially less expensive, as the populations most at risk are often in very low socio-economic regions. In addition, a new method should also improve on the time taken to analyse a sample. When using ICP-MS, as mentioned above, samples need to be transported to a laboratory for analysis, which increases not only the cost of analysis but also the length of time between the patient visit and the diagnosis. Therefore, a new technology should also be readily deployable in the field and have a short analysis period.

1.5.3 Detection of Arsenic in Human Nails by X-ray Fluorescence

The use of x-ray fluorescence (XRF) to detect As in human biological samples is still in its infancy. The concept of using XRF to detect As is based on the

fact that when an atom (e.g. As) absorbs a photon, an electron is ejected from the inner shell, which leads to the emission of an x-ray of a known energy, which is specific for each element (approximately 10.5 keV for As). Since each x-ray is specific for each element, and thus XRF is able to distinguish one element from another (Studinski, McNeill, Chettle, et al., 2005).

Presently, the only published data that aims to use XRF to detect As in human biological samples are several papers using "phantoms" as surrogates to human tissue (Fleming & Gherase, 2007; Roy, Gherase & Fleming, 2010; Studinski et al., 2005). Phantoms are created using polyester resins that are made to mimic, for example, human skin or nail. A known amount of As, or any element, is added to the phantom prior to induction of XRF. After inducing XRF, the known concentration of the phantom is compared to the detected signal. Fleming et al. (2007) reported an XRF procedure capable of detecting As at concentrations of 0.446 ± 0.006 μg/g after 120 seconds of exposure time (Fleming et al., 2007). However, there are no reports of validation of this test method in human biological samples.

The use of an XRF technique to measure [As] in human biological samples presents several advantages. An XRF method requires only approximately 120 seconds for a sample to be run (though in the following thesis work, samples were each measured 5 times to obtain maximum accuracy), followed by approximately 15 minutes of data analysis. Conversely, it takes approximately 5 minutes to run a similar sample through ICP-MS, but this does not take into account the time required to ship samples from the collection location to a laboratory or preparation

of the sample prior to analysis (up to 2 hours for sample preparation, as described later). As well, with no reagents required, the cost to run each sample is reduced for XRF in comparison to ICP-MS. The XRF apparatus used in this study costs $50,000 CND, with an approximate sample processing cost of $10, resulting in both [As] and [Se]. In comparison, a standard ICP-MS costs $250,000 CND, with an approximate sample processing cost of $10 for a single element, with that cost increasing to $15 for multi-element analysis. Finally, the XRF device is battery operated and readily deployable in the field, so remote areas that are far from a traditional and well-stocked laboratory would become more available to testing. In contrast, an ICP-MS requires a constant energy source and sterile laboratory environment to function properly. While the XRF technique is currently limited to detecting the total amount of As in a biological sample, in the future, it may be capable of also detecting the concentrations of As species. A method called synchrotron x-ray Absorption Near Edge Structure (XANES) is currently able to achieve these As species results. However the XANES requires the generation of synchrotron radiation, a very high intensity x-ray beam contained in a large machine, and it requires the ability to finely tune the energy range of interest (Meunier et al., 2010). The hope is that this method can be scaled down to a more deployable instrument, though this would probably be more expensive, at least initially, than ICP-MS methods or the XRF method discussed in this thesis.

1.6 Research Objectives

This thesis has 4 main research objectives, examined in 4 separate chapters. These chapters are followed by a summary chapter (chapter 6) that explains the

main results of each research objective, and how the results relate to each other, along with recommendations for future research. The following research objectives were tested based on information from: a) a questionnaire administered to 179 people on their eating and drinking habits over a 3 day period, as well as key social and demographic information, and b) water and biological samples (urine, hair, fingernails, and toenails) analyzed for [As] by ICP-MS and XRF.

1.6.1 Objective 1

To determine which factors and biological samples were associated with the estimated amount of arsenic consumed via water, food, and a combination of both

Multiple linear regression and multiple logistic regression analyses were used to evaluate the associations between biological samples and estimated consumption of As from water and food, while accounting for household and participant characteristics, based on questionnaire data. Determining which biomarkers (urine, hair, toenails or fingernails) were associated with a high level of As consumption increased our knowledge of the usefulness of biomarkers.

1.6.2 Objective 2

To determine which factors predicted the concentration of urinary arsenic metabolites

Permutation analyses were used to determine statistical differences in urinary As metabolites between locations, gender and exposure groups (based on a

10 μg/L water [As]). Linear and logistic regression analyses were used to determine which household characteristics, and participant demographic and food and water consumption information, was most influential in predicting the concentration of various As metabolites in urine. Understanding which factors are involved in predicting high concentrations of urinary As metabolites may be used to decrease the exposure of participants to As and to further our knowledge of As metabolism in humans.

1.6.3 Objective 3

To determine which factors predicted the total concentration of arsenic in human fingernail, toenail, and hair samples

Multiple linear and logistic regression analyses were used to determine which demographic, biologic, and consumption information was most influential in predicting the total [As] in fingernails, toenails, and hair. Determining which factors most influence the [As] in a biological sample will help to elucidate which biological sample may be most useful for different types of future epidemiological studies.

1.6.4 Objective 4

To evaluate the performance of a novel method of arsenic and selenium detection in human nail samples by XRF in comparison to ICP-MS, in a population exposed to naturally occurring As in drinking water

Fingernail and toenail samples collected from study participants were analyzed for total As and Se concentrations by both ICP-MS and XRF techniques. Comparison of results by concordance correlation coefficients, kappa analyses, sensitivity and specificity analyses, and linear regression analyses determined the suitability of the XRF technique for use on human nail samples.

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2 Understanding Self-Reported Exposure to Arsenic of a Sample Population from Areas of Nova Scotia at Risk of Arsenic Exposure

2.1 Abstract

Several different biological samples – including urine, fingernails, toenails, and hair – can be used for estimating arsenic (As) exposure. The goal of this study was to determine which biosamples and demographic and other factors were associated with short-term As ingestion. These biosamples were collected from 179 individuals from two locations in Nova Scotia, Canada. Each sample, and the participant's home drinking water, was analyzed by inductively coupled plasma-mass spectrometry for the total [As] (water As range: < 0.017 – 309.2 μg/L). A questionnaire was used in face-to-face interviews to estimate As exposure via water and food consumption over the 3 days prior to the interview, along with other demographic, lifestyle, and household characteristics. Linear regression analyses found that specific-gravity (SG) adjusted urinary [As] (range: <0.355 – 661.1 μg/L) was significantly associated with As ingested from water, while logistic regression analyses found that high levels of SG-adjusted urinary [As] were significantly associated with increased odds of being in the high exposure categories of food As consumption and total As ingestion, by up to 9.4 (95% CI: 2.8 – 31.8) and 10.3 (95% CI: 2.8 – 37.3) times, respectively. Fingernail [As] and water pH were also significantly positively associated with water As consumption Income was significantly associated with high food As consumption, while water well depth was associated with high total As ingestion. Neither toenail nor hair [As] was

significantly associated with As consumed from water, food, or both, and therefore should not be utilized for determining short-term As exposure.

2.2 Introduction

Arsenic can be found in many different chemical arrangements and is most often found in water in the inorganic trivalent (arsenous acid, H_3AsO_3) and pentavalent forms (H_2AsO_4 and $HAsO_4^-$) (Agency for Toxic Substances and Disease Registry (ATSDR), 2007). It is now well known and universally accepted that As can be extremely hazardous at high concentrations, and recently the World Health Organization reduced the maximum contaminant level (MCL) of As in drinking water by eighty percent, from 50 μg/L to 10 μg/L (World Health Organization (WHO), 2008). There have been many epidemiological investigations into the health effects of As, resulting in clear evidence that chronic exposure to high [As] can lead to a greater risk of different types of cancers, including: skin, kidney, urinary bladder, and lung (Banerjee et al., 2008; Chiou et al., 2001; Ferreccio et al., 2000; Guo, Chiang, Hu, et al., 1997; Kurttio, Pukkala, Kahelin, et al., 1999; Yu, Liao & Chai, 2006). In these studies, exposure to As was estimated by determining the [As] in drinking water, with or without taking water consumption habits into account. Additionally, it is common for a single test of water [As] to be assumed to be stable and representative of a well-water [As] for over a decade of past exposure in some studies. When As is ingested through a water source, the body is able to metabolize the inorganic As, through a series of reduction and methylation steps, into an organic form. Pentavalent As (arsenate, As(V)) is reduced to trivalent As (arsenite, As(III)), which is then methylated to form monomethylarsonic acid

(MMA(V)). MMA(V) is reduced to form monomethylarsonous acid (MMA(III)), which is methylated to dimethylarsinic acid (DMA(V)). Finally, DMA(V) is further reduced to produce dimethylarsinous acid (DMA(III)) (Cullen, McBride & Reglinski, 1984). Long thought to be detoxified metabolites, organic arsenicals have recently been shown to produce genotoxic effects (Kitchin, 2001; Kligerman et al., 2003; Thomas, Styblo & Lin, 2001). Although a maximum [As] of 10 μg/L in water is recommended by the World Health Organization, many areas of the world have neither the knowledge nor the resources to assess the quality of their drinking water. Areas of particular concern are low socio-economic regions of India, Bangladesh, Mongolia, and Taiwan, and certain areas of China and South America, as well as others.

Many areas of Nova Scotia, Canada, have [As] in groundwater which are above the 10 μg/L guideline, which is due both to natural rock deposits as well as human activities. For example, large areas of soil and groundwater have been contaminated by As surrounding retired gold mines in the Montague Mines area, and in the areas surrounding the Sydney tar ponds (Meunier et al., 2010). These contaminated areas are concentrated sources for As to leach into underground waterways, which can allow the dispersal of As over large areas and into private wells used for drinking water (World Health Organization (WHO), 2008).

As of 2010, the province of Nova Scotia was reported by the Canadian Cancer Society to have the highest age-standardized incidence rates of cancer in males for the last 5 years, and in females for the last 3 years (Canadian Cancer Society, 2010). In particular, Nova Scotia residents suffer from the highest rates of

bladder, kidney and lung cancer in the country. Even with this information, few epidemiological studies have been performed in Nova Scotia to assess potential linkages between exposure to arsenic-contaminated drinking water and cancer incidence.

Assessing the degree to which people have been exposed to As can be achieved in several ways. The most obvious method is to analyze a person's drinking water for the presence of As, which can be accomplished by use of an inductively coupled plasma mass spectrometer (ICP-MS) (United States Environmental Protection Agency, 1994). After determining how much As is present in a person's water supply, investigation of the amount of this water (and other liquids mixed with this water, or foods cooked or processed with this water) that is consumed will estimate an average daily consumption of As. Although this method can estimate As exposure from a personal water supply, it is not able to capture any exposure to As that may occur outside of the home, unless other water sources outside the home are tested as well. Once ingested and metabolized by the body, most As is rapidly excreted in the urine. In a human experiment, it was found that after a single 500 μg dose of sodium arsenite, approximately half of the As excreted in urine over a 4-day period was excreted in the first 28 hours. After this 28-hour mark, urinary [As] steadily tapered off (Buchet, Lauwerys, & Roels, 1981).

Due to its ability to create strong sulfhydryl bonds, As binds preferentially with keratin and therefore As is also incorporated in small amounts in hair and nails (Hopps, 1977). Using ICP-MS, the concentration of total As in water, hair, nails and urine samples can be determined. Additionally, using High Performance Liquid

Chromatography, individual As species in water and urine samples can be identified and subsequently quantified using ICP-MS. Although studies have used many biological markers as a surrogate for As exposure, it remains unclear how appropriate these biomarkers are for this purpose. While a suite of potential biomarkers have been used in the past, identifying which is most appropriate for use on short-term exposure estimates will focus attention and resources towards that biological sample, and facilitate more productive and efficient studies in the future.

The objective of this study was to investigate the relationship between short-term As ingestion from different sources (water, food, and total) and several biological samples, to determine which biomarker is most appropriate to collect in future studies when a surrogate of short-term As consumption is needed.

2.3 Materials and Methods

2.3.1 Screening Procedure to Identify a Sampling Frame and Study Groups

In June of 2010, a total of 3,157 packages were delivered to all households in two rural communities in Nova Scotia (NS), Canada. Canada Post was contracted to deliver 1,597 packages to Hubbards, approximately 50 km southwest of Halifax, NS, and 1,560 packages to Fall River, approximately 25 km north of Halifax, NS. One community, Fall River, is found in a gold district that has homes with a risk of having high [As] in drinking water and soil. The second community, Hubbards, is not in a gold district, but due to a geological anomaly, has homes with a risk of having high [As] in drinking water, but relatively low [As] in soil. These communities were selected based on consultations with the Ministry of the

Environment of Nova Scotia who have maps identifying “high risk” areas of As in drinking water, based on previous sampling (See Appendix A).

Packages contained the following:

- information on the proposed research study and intended uses of data;
- a coded, 50ml, trace metal-free centrifuge tube (Corning) for water sample collection;
- detailed instructions on the proper technique to collect a water sample;
- a questionnaire to determine participant eligibility and to collect specific contact information;
- a pre-paid, coded, pre-addressed envelope for the questionnaire and contact information, to be sent to the University of Prince Edward Island; and
- a pre-paid, coded, pre-addressed plastic envelope for the water sample to be mailed to Health Canada.

Initial eligibility criteria required that: 1) the participants drank their own tap water on a daily basis; 2) the tap water came from a private, drilled well; 3) the participants lived in their home for at least 3 years; and 4) the participants were between the ages of 25 and 75. Eligibility criteria 1 and 2 were included because we wanted to be certain that any potential participants would be exposed to As via

their drinking water, if there were As in that water. People who didn't drink their water, or obtained their water from a regulated water supply (i.e. from non-rural environments) were not of interest to this study. Requiring participants to have lived in their home for at least 3 years, eligibility criteria 3, was included for a different portion of the study, in which biological markers of genetic damage were collected, analyzed, and related to As exposures, but this portion is not discussed in this thesis. The 3-year criterion was chosen to ensure that any potential As-related genetic damage effects would have had ample time to manifest in participants. Finally, an age restriction of between 25-75 years was chosen so that results could be easily compared to other studies with the same age group (Health Canada, 2010).

According to instructions on the proper method of water collection, the participants mailed a well-water sample, in the pre-paid and pre-addressed envelope provided, to Health Canada for processing. Eligible water samples were then sent to either Maxxam Analytics Laboratory (Dartmouth, NS) or Acadia University (Wolfville, NS) for total As quantification via inductively coupled plasma mass spectrometry. Minimum detection limits (MDLs) for determining As concentrations in water at Maxxam Analytics and Acadia University were 1 and 0.14 μg/L, respectively. One laboratory could not handle the volume of samples expected for this screening procedure.

All homes with an elevated [As] in their tap water were invited to participate in the study to maximize the number of participants with elevated As exposure from drinking water. A random sample of homes with water [As] below

the MCL were selected from those that met the inclusion criteria, stratified on community to ensure relatively equal numbers of homes and participants among communities. Random samples were based on following a list of randomly assigned ID numbers, which were found not to have any bias of gender, age, water [As], street address or any other factors. Homes that were below the MCL were contacted in order of randomly assigned ID numbers. The homes below the MCL chosen to participate in the study were representative of the total number of returned water samples in terms of water [As]. For instance, if 20% of all collected water samples below the MCL were between 5 – 7 μg/L, then approximately 20% of participants chosen to participate in the study were from this water [As] category, and so on for all [As] from <MCL to 10 μg/L.

The total number of homes and participants included in the study was partly dependent on sample size calculations and partly on budgetary constraints. The following assumptions were utilized for a sample size calculation: 95% confidence and 80% power; 35% prevalence of genetic damage in Fall River; and 15% prevalence of genetic damage in Hubbards. An *in vitro* study from Mongolia (Feng et al., 2001) found that 15% of a low exposure group had genetic damage at the average As exposure concentration of 4.4 ± 1.0 μg/L in water, while 89% of the high exposure group had evidence of genetic damage, with an average As exposure concentration of 527.5 ± 23.7 μg/L. From a study in Chile (Moore et al., 1997), a strong correlation was found between creatinine-adjusted urinary [As] (range of 4 to 1,893 μg/L) and water [As] (range of 13 to 670 μg/L), and the group with 53.9 to 137.3 μg/L of As in urine had 2.1 times the prevalence of genetic damage compared

to the lowest exposure group with ≤ 53.8 μg/L of As in urine ($p < 0.05$). Our assumption of 35% prevalence of genetic damage in Fall River seemed reasonable, considering, in reference to the Mongolian and Chilean studies mentioned above, genetic damage in 15% of a low exposure population, multiplied by 2.1, is 31.5%, our expected genetic damage prevalence for high exposure groups, assuming a linear relationship. Using these assumptions, at least 71 participants in each community were required, or 142 in total, which we rounded up to 150 participants in total being appropriate for determining a statistically significant difference in genetic damage between the participants of the two communities.

2.3.2 Sample Collection

In July of 2010, 108 homes were visited for further data and sample collection. In these homes, 179 persons were found to meet the inclusion criteria and formed the study population, 72 people (40.2%) were sampled from the Fall River area, while 107 people were from the Hubbards area. A higher number of participants were from Hubbards due to additional low exposure participants being recruited for a larger low exposure group, for comparison purposes (more likely to be obtained from Hubbards than Fall River). This larger low exposure group would thus include two sub-groups, those with water [As] below the MDL, and those in between the MDL and the MCL. The participants were given the option to have the interview and sample collection performed either at their residence or at a nearby mobile laboratory. All of the participants opted to perform the interview and sampling at their home.

For each participant, there was a face-to-face interview conducted by a trained graduate student from the Atlantic Veterinary College at the University of Prince Edward Island. The interview consisted of a questionnaire designed to collect information from participants about their eating and drinking history during the previous 3 days, smoking habits, and socioeconomic information, as well as other demographic and activity information (see Appendix B).

At the time of the home visit, a second water sample was collected, and a public health nurse was responsible for collecting biological samples from each participant. These samples included urine, fingernails, toenails, hair, and a cheek swab, and each non-liquid sample was placed into a separate, labeled, zip-lock bag. Urine was collected in a 100ml trace metal free bottle (Fisher Scientific). For urine collection, participants were instructed to initially void a small amount of urine into the toilet before beginning their sample collection in the bottle, if possible. Hair samples were collected from the nape of the participant's neck, if there was sufficient hair to collect. Tools used to collect samples, such as nail clippers and scissors, were disinfected using sterilizing alcohol swabs between each participant.

After collection, samples were put on ice until they arrived at the mobile laboratory; this time was never more than 3 hours, and was most often approximately 45 minutes. The mobile laboratory was set up by the research team in the area for sample pre-processing to get them ready for shipment to the laboratories where they would undergo analyses. Finger and toenails, hair, urine and household tap water were each tested for the total [As]. From the questionnaire, we were able to determine how much of the home tap water the

participants had consumed over the last 48 hours. An estimate of daily intake of As from water was calculated by multiplying a participant's daily water consumption (water, tea, coffee, juice, foods boiled in water, etc.) by the [As] in their tap water. For example, if a person consumed 1.5L of water in a day, and that water had an [As] of 25 µg/L, that participant's estimated daily intake of As from water would be 1.5L * 25 µg/L = 37.5 µg of As per day.

In addition, participants were asked how much chicken, rice and seafood they had eaten in the last 3 days. These foods have been found to have more As in them relative to other foods, and they are therefore important to take into account when calculating daily exposure to As (Dabeka et al., 1993; Schoof et al., 1999). Estimated As ingestion from food was calculated using reference values from Dabeka (1993) and Schoof (1999). Seafood was given an assumed [As] of 2.36 µg/g, chicken was given 0.86 µg/g, and rice was given 0.303 µg/g (where 250 ml of dry rice is equal to 195.5g). All food [As] referred to uncooked foods. To obtain the total amount of As consumed from these foods, the [As] was multiplied by the amount of food consumed during the last 3 days:

$$Estimated\ As\ consumption\ from\ food = \ food\ consumed \ \times \ food\ [As](\frac{ug}{g})$$

In addition to uncooked estimates, estimates of As consumed from prepared (boiled) rice were calculated. Dry rice was assumed to have a moisture value of 9.70g per 100g of uncooked rice (white, long grain) and cooked rice was assumed to have a moisture value of 70.36g per 100g of cooked rice (Government of Canada, 2002). Using these values, a moisture difference of 60.66g was calculated between raw and cooked rice. The amount of water absorbed by rice, 60.66g per 100g

consumed, was then divided by 100 to represent the water absorbed per 1g of rice (0.6066g). This value was multiplied by the amount of rice consumed, in grams, by each individual (where 250ml of cooked rice is equal to 184.9g). Finally, this product was multiplied by the [As] of the water that was used to boil the rice, and added to the amount of As present in the dry rice prior to cooking, resulting in the following formula:

$$Arsenic\ consumed\ from\ rice = \quad water\ absorbed\ by\ rice \quad 0.6066g \quad \times$$

$$rice\ consumed \quad g \quad \times\ water \quad As \quad \frac{\mu g}{1000g} \quad + \quad As \quad in\ dry\ rice\ (0.303 \quad \frac{\mu g}{g} \quad \times$$

$$dry\ rice\ consumed \quad g$$

For foods that were given an assumed concentration of As (rice, seafood, and chicken), it is important to keep in mind that though the assigned As concentrations were taken from peer-review sources, these are still rough estimates. Food grown in different regions or with different fertilizers can have different As concentrations, and without sampling and analyzing each food source for its As concentration, the assigned As concentrations in each rice, seafood, and chicken are, necessarily, estimates.

2.3.3 Laboratory Analysis

After collection and pre-processing, water and biological samples were sent to Acadia University, in Wolfville, Nova Scotia, for ICP-MS analysis. The minimum detection limit for the ICP-MS for urine, fingernails and toenails, hair, and water are shown in Table 2.1. Water and urine MDLs were determined using the United States Environmental Protection Agency guidelines for determination of trace

elements in water and wastes by ICP-MS (Method 200.8, revision 5.4) (US EPA, 1994), and was calculated as MDL=t*s, where t=3.14 (student's t value at 99% confidence level) and s is the standard deviation (in μg/L) for 7 replicate measurements of a standard near the detection limit. For fingernails, toenails, and hair, a digestion process is required to dissolve the As into a liquid media (water). Therefore, MDLs were calculated as MDL=t*s*20ml, where the limit of detection is three-times the standard deviation of a blank sample, and 20ml is the volume of the digested sample. A sample mass of 5mg and 10mg was used for nail and hair digestion, respectively, as recommended (US EPA, 1994).

2.3.4 Statistical Analyses

Data collected at the participant and household levels were compared between both locations and exposure categories (exposed or unexposed), to identify any significant differences. Due to non-normality in many of the collected variables (tested using the Shapiro-Wilk W test) continuous variables (water and biological samples, consumption habits, etc.) were analyzed for statistical differences in median values using permutation analysis (Baglivo, 2005) with 10,000 resampling events (permutations), and categorical data were tested using Wilcoxon Rank Sum Test. Binomial data were tested for differences in distribution between locations, genders, and exposure groups, using Pearson's chi-square analysis. For some of the collected biological samples, the [As] was below the MDL, and therefore, when analyzing the data, such data points were given the value of one-half of the sample minimum detection limit.

Table 2.1 Minimum detection limits for biological samples using ICP-MS

Sample	MDL* (μg/L)
Water	0.017
Urine	0.709
Fingernail	0.153
Toenail	0.153
Hair	0.068

*Minimum detection limit
ICP-MS: inductively coupled plasma-mass spectrometry

Linear regression models were fitted using daily water As consumed, which was logarithmically transformed (natural log) to comply with the normal distribution requirement for this analysis. Interpretation of coefficients from a natural-log transformed linear regression model on a non-logarithmic scale requires the back-transformation of regression model parameter estimates, using the formula e^x. Using this formula, the model coefficients are no longer in the original units, μg in this case, but are in percent change compared to a baseline. For ease of interpretation, and for comparability of different models within and between chapters, continuous variables in log-transformed linear regression models will be interpreted as the percent change in the outcome variable that occurs when increasing the continuous independent variable value from the 25th percentile to the 75th percentile (i.e. the interquartile range). For categorical variables, all percent changes in the outcome variable will be interpreted with respect to the baseline category of the independent categorical variable.

Data for As consumption from food sources were strongly right-skewed and they could not be transformed using logarithms (even after scaling and centering the variable), square roots or cube roots to fit a normal distribution. For this reason, As consumption from food was modeled using logistic regression with a cut-point chosen from the midpoint of the consumption distribution, 10 μg. For the total amount of As consumed from water and food combined (also not normally distributed, even with transformations), logistic regression was also utilized, with the cut-point chosen for the "exposed" population from the mid-point of the total

As consumption data, 88 μg. Participants who consumed less than the cut-point were placed in the "unexposed" category.

Due to varying urine concentrations of different participants, urine [As] values were scaled based on each sample's specific gravity (SG) value, which was measured by calculating its refractive index. Urine [As] values were scaled using the following formula:

$$Specific\ Gravity - Adjusted\ Urinary\ \ As\ \ \frac{ug}{L} =$$

$$Urine\ \ As\ \ \frac{ug}{L}\ \ * [\frac{mean\ SG-1}{sample\ SG-1}]$$

In this adjustment, samples with low SG values (dilute samples) have [As] adjusted upward, while samples with high SG values (concentrated samples) have [As] adjusted downward. In this way, fluctuations in sample concentration, void number per day, and time of void are partially controlled for across the sample population (Nermell et al., 2008; Suwazono, Akesson, Alfvén, et al., 2005).

All linear regression models were tested for assumptions of linearity (visually: standardized residuals vs. inverse normal curve; statistically: Shapiro-Wilk W test for normal data) and homoscedasticity (visually: residuals vs. fitted values plot; statistically: Cook-Weisberg test for heteroscedasticity). Logistic regression models were tested for goodness of fit (statistically: Pearson chi-squared and Hosmer-Lemeshow goodness of fit test) and evaluated for influential covariate patterns (standardized residuals vs. covariate pattern plot; standardized residuals outside of ± 3), high leverage points (Pearson residuals vs. leverage plot; >30% of mean value), and delta-betas (Pearson residuals vs. delta-beta plot).

Potential variables for inclusion in models were chosen first by simple association with the dependent variable at a p-value cut-off of 0.15. Variables in final models required a p-value of $p < 0.05$ to be included as an explanatory variable. Final models were constructed using manual step-wise inclusion.

All statistical analyses were performed using Stata 11 (StataCorp LP, College Station, Texas).

2.4 Results

2.4.1 Descriptive Statistics of Participants' Home Characteristics

A total of 784 water samples were returned, corresponding to a response rate of nearly 25% (387 from Hubbards, 397 from Fall River). Of these, 676 (or 86% of returned samples) eligible water samples were analyzed for the presence of As. The remaining water samples were not analyzed due to lack of accompanying information (i.e., address, phone number, etc.) or ineligibility. Eighty-two water samples (12%) were above the recommended MCL for As in drinking water, set by Health Canada and the World Health Organization, of 10 μg/L (48 from Hubbards, 34 from Fall River). Of the total number of participants who returned water samples, 108 homes were contacted and enrolled in the study, 67 from Hubbards and 41 from Fall River (14%), of which 45 (42%) were above the MCL and 63 (58%) were below the MCL.

Participants were split into two groups: exposed (water [As] of $\geq$ 10 μg/L) or unexposed (water [As] of <10 μg/L). Descriptive statistics of water [As] and other

demographic and consumption information, dichotomized into location and exposure categories, are presented in Tables 2.2 to 2.7.

In the sample population from the Hubbards area, over 95% of participants reported owning their homes. Participants were nearly evenly spread across the categories of reported time living at their current home; 23% reported living in their current home for between 3-5 years, 16% between 5-10 years, 27% between 10-25 years, and 34% for more than 25 years. However, over 53% of participants reported living in the community or general area for more than 25 years, with only 27% of participants living in the community for less than 10 years.

In the Fall River area, the vast majority of participants, nearly 99%, reported owning their home. Once again, there was a relatively even spread of individuals across the "time in home" categories. Again, the majority of participants (41%) had lived in the community for more than 25 years, and 43% had lived in the community for less than 10 years.

The majority, 60%, of the participants reported a yearly income of > $50,000 (category 3), 23% of participants reported earnings between $30,000 and $50,000 per year (category 2), and the remaining 17% reported earnings of less than $30,000 per year (category 1). Nearly 78% of the participants from Fall River reported a yearly income of greater than $50,000 (category 3), with only 2% reporting an income of <$30,000 per year (category 1), leaving 20% reporting an income of between $30,000 - $50,000 per year.

Table 2.2 Summary of collected home level data (continuous variables), by location

	Hubbards		Fall River		
Variable	**Summary parameter**	**Range**	**Summary parameter**	**Range**	**p-value[1]**
n	67		41		
H_2O [As] (µg/L) (mean, 95% CI)	23.1 (10.6 – 36.0)	0.009[2] - 309.2	15.0 (8.0 – 22.1)	0.009[1] - 92.4	0.446
H_2O [As] (µg/L) (median, IQR)	3.2 (0.2 – 20.7)		5.4 (0.3 – 20.2)		0.501
Own Home (Y/N)	95%		99%		0.602
H_2O Softener Use (Y/N)	**13%**		**35%**		**0.001**
H_2O Treatment (Y/N)	23%		33%		0.074
Well Depth (m) (mean, 95% CI)	49.7 (40.9 - 58.3)	1.8 - 152.4	63.6 (51.3 - 76.1)	4.6 - 137.2	0.133

[1]Bolded data represent variables that are significantly different ($p < 0.05$) between locations
[2]Data represent half of the minimum detection limit of ICP-MS for water samples (0.017 µg/L)
IQR = interquartile range; 95%CI = 95% confidence interval

Table 2.3 Summary of collected home level data (categorical variables), by location

Variable	Hubbards % in each category	Fall River % in each category	p-value[1]
Annual Income Category			**0.012**[2]
< $30,000	**17%**	**3%**	**0.028**
$30,000 - $50,000	24%	15%	0.318
> $50,000	**59%**	**82%**	**0.019**
Time in Home Category			0.374[2]
3-5 years	25%	28%	0.810
5-10 years	15%	30%	0.063
10-25 years	30%	15%	0.085
>25 years	30%	27%	0.796
Time in Community Category			0.223[2]
3 – 5 years	13%	15%	0.822
5-10 years	**15%**	**33%**	**0.033**
10-25 years	21%	10%	0.147
>25 years	51%	42%	0.411

[1]Bolded data indicate variables that are significantly (p-value $\leq$ 0.05) different between categories
[2]Indicates overall p-value for categorical variable

Table 2.4 Summary of collected home level data (continuous variables), by water As exposure group

	Unexposed		Exposed		
Variable	**Summary Parameter**	**Range**	**Summary Parameter**	**Range**	**p-value[1]**
n	63		45		
H_2O [As] (µg/L) (mean, 95% CI)	1.6 (1.0 - 2.3)	0.009[2] - 9.4	46.1 (28.7 – 63.5)	10.3 - 309.2	
H_2O [As] (µg/L) (median, IQR)	**0.3 (0.2 – 1.3)**		**22.2 (15.5 – 47.6)**		**<0.001**
Own Home (Y/N)	94%		100%		0.082
H_2O Softener Use (Y/N)	19%		26%		0.405
H_2O Treatment (Y/N)	34%		17%		0.056
Well Depth (m) (mean, 95% CI)	**46.2 (37.3 - 55.9)**	1.8 - 137.2	**67.0 (56.7 - 77.3)**	4.6 - 152.4	**0.005**

[1]Bolded data indicate variables that are significantly (p-value $\leq$ 0.05) different between categories

[2]Data represents half of the minimum detection limit of ICP-MS (0.017 µg/L)

IQR: interquartile range, 95%CI= 95% confidence interval

Table 2.5 Summary of collected home level data (categorical variables), by water As exposure group

Variable	Unexposed % in each category	Exposed % in each category	p-value
Annual Income Category			0.062[1]
< $30,000	14%	7%	0.301
$30,000 - $50,000	25%	15%	0.231
> $50,000	61%	78%	0.082
Time in Home Category			0.084[1]
3-5 years	24%	30%	0.509
5-10 years	17%	25%	0.345
10-25 years	24%	25%	0.888
>25 years	35%	20%	0.106
Time in Community Category			0.564[1]
3 – 5 years	16%	11%	0.511
5-10 years	17%	27%	0.226
10-25 years	18%	16%	0.834
>25 years	49%	46%	0.704

[1]Indicates overall p-value for categorical variable

Wilcoxon Rank Sum analysis revealed that participants in Fall River had a higher median income than in Hubbards ($p = 0.011$). There were no significant differences between locations for any other home level variables.

When comparing exposure groups, permutation analysis showed that the exposed group had significantly deeper median well depth ($p = 0.005$), and Wilcoxon rank sum tests showed significantly more homes in the unexposed group employed some type of water treatment system ($p = 0.056$). A higher average income, that was nearly significant ($p = 0.062$), was found in the exposed category, and this group had also lived in their current home longer, although not significantly, compared to the unexposed group ($p = 0.083$). Because of the intentional grouping of water samples above and below the MCL of 10 μg/L, exposed and unexposed groups were also found to have significantly different [As] in their water ($p < 0.001$).

Water [As] was not significantly different between locations (p-value 0.501). In Hubbards, 26 water samples out of 67 (39%) were above the MCL of 10 μg/L, while 19 of 41 water samples from Fall River (46%) were above the MCL. Overall, 45 out of the 108 well samples were found to have greater than or equal to 10 μg/L of As (42%). However, the sampling scheme dictated that we enroll all willing participants in homes with [As] above 10 μg/L and then a representative sample of participants in unexposed homes from each community. When only samples above the MCL are compared, locations were still not shown to have significantly different median As values ($p = 0.114$), with medians of 35.0 μg/L and 20.9 μg/L for Hubbards and Fall River, respectively. Histograms of water [As] for water

samples greater than the MCL are presented in Figure 2.1, by each location. A wide range of water [As] was found, from a minimum of below the detection limit of the ICP-MS (0.017 μg/L) up to 309 μg/L; over thirty-times the recommended maximum concentration limit of As in drinking water.

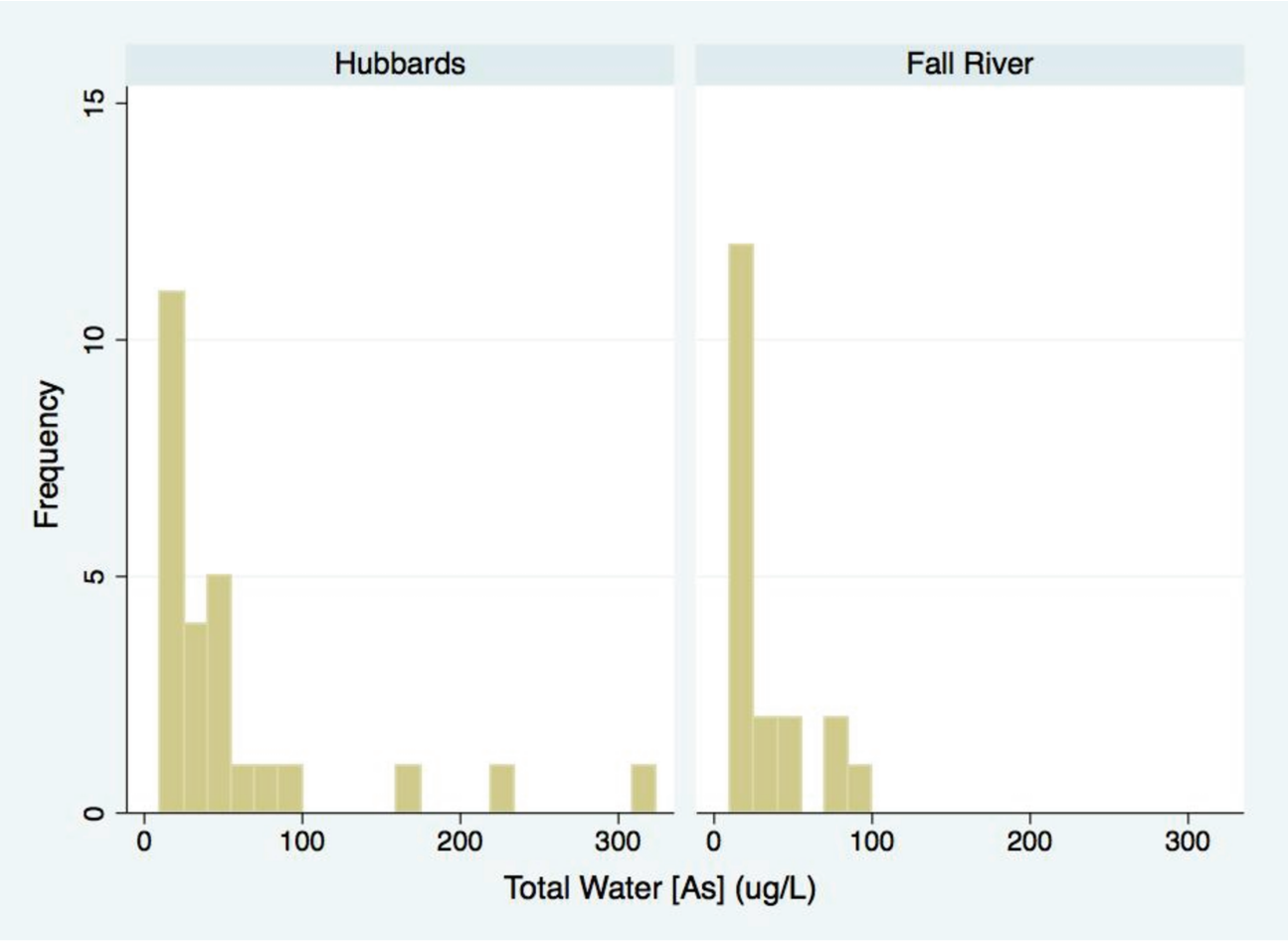

Figure 2.1 Histogram of total arsenic concentrations of water samples which were above the maximum contaminant limit of 10 μg/L, by location

2.4.2 Descriptive Statistics of Demographics and Consumption Patterns of Participants

Detailed descriptions of the participant level demographics and eating and drinking history data are presented in Table 2.6, by location, and in Table 2.7, by water As exposure group. In the Hubbards location, the sample population consisted of 107 individuals (46 males, 61 females) from 67 homes, with a mean age of 58 years (60 years for men, 57 years for women). In comparison, in the Fall River area, the sample population consisted of 72 individuals (34 males, 38 females) in 41 homes, with a significantly lower mean age of 54 years (55 years for men, 54 years for women), compared to Hubbards.

Table 2.6 Summary of participant level questionnaire data on demographics and characteristics, by location

	Hubbards		Fall River		
Variable	**Summary Parameter**	**Range**	**Summary Parameter**	**Range**	**p-value[1]**
n	107		72		
Age (years) (mean, 95% CI)	**57.7 (55.1 - 60.4)**	28 - 78	**54.9 (51.8 - 58.0)**	34 - 83	**0.016**
Education Category (% of population in each category: 1, 2, 3 & 4)[2]	1 – 7%, 2 – 38%, 3 – 20%, 4 – 35%		1 – 3%, 2 – 33%, 3 – 18%, 4 – 46%		0.095
Estimated H_2O Consumed (L / 2 days) (mean, 95% CI)	2.5 (2.2 - 2.8)	0 - 7.7	2.2 (1.8 - 2.6)	0 - 7.2	0.570
Rice Consumed (Y/N)	12%		21%		0.117
Estimated Rice Consumed (cups /3 days) (mean, 95% CI)[3,4]	2.0 (1.0 - 3.0)	1 - 7	1.0 (1.0 -2.0)	1 - 3	1.000
Seafood Consumed (Y/N)	**31%**		**46%**		**0.041**
Estimated Seafood Consumed (ounce / 3 days) (mean, 95% CI)[3,4]	7.9 (6.0 -10.0)	1 - 24	7.2 (5.0 - 9.0)	1 - 22	1.000
Chicken Consumed (Y/N)	56%		51%		0.537
Estimated Chicken Consumed (ounce / 3 days) (mean, 95% CI)[3,4]	5.1 (4.0 - 6.0)	1 - 16	5.9 (5.0 - 7.0))	1 - 16	0.603
Estimated As Consumed from Rice, Seafood and Chicken (µg) (median, IQR)[4]	7.0 (2.3 - 341.5)	0 – 5,105.2	168.4 (4.7 – 670.2)	0 – 5,151.6	0.140

[1]Bolded data indicate variables that were significantly (p-value ≤ 0.05) different between categories
[2]Education categories: 1 – primary, 2 – secondary, 3 – college, 4 – university
[3]Refers only to participants who consumed that product
[4]Rice [As] = 0.303 µg/g, chicken [As] = 0.0864 µg/g, seafood [As] = 2.36 µg/g
IQR: interquartile range, 95% CI = 95% confidence interval

Table 2.6 Summary of participant level questionnaire data on demographics and characteristics, by location (continued)

	Hubbards		Fall River		
Variable	**Summary Parameter**	**Range**	**Summary Parameter**	**Range**	**p-value**
Estimated As Consumed from Water (μg / 2 days) (median, IQR)	3.2 (0.4 - 52.1)	0 - 1,798.8	4.0 (0.3 - 33.7)	0 - 133.1	0.476
Estimated Total As Consumed (μg / 3 days) (median, IQR)	64.3 (9.3 – 421.7)	0 – 5,260.7	202.6 (6.3 – 690.7)	0 – 5,192.2	0.099
Estimated Other Foods Consumed (cups / 3 days) (median, IQR)[1]	1.0 (0 - 2.0)	0 - 12	0.5 (0 - 2.0)	0 - 4	0.857
Antacid (Y/N)	28%		19%		0.548
Antibiotics (Y/N)	0%		0%		0.097
Prescription Medication (Y/N)	4%		0%		0.659
Smoker (Y/N)	18%		11%		0.100
Estimated Smoking Amount (cigarettes/day) (mean, 95% CI)[2]	14.0 (10.0 - 18.0)	2 - 25	9.0 (8.6)	1 - 25	1.000
Estimated Bathing/Showering (times/week) (mean, 95% CI)	6.0 (1.6 - 10.4)	0 - 14	6.4 (3.2 - 9.6)	2 - 10	0.052
Use Shampoo with Selenium (Y/N)	6%		7%		0.788
Dye Hair (Y/N)	29%		36%		0.315

[1]Foods boiled in tap water or which used water to prepare (cooked vegetables, soups, pasta, etc.)
[2]Refers only to participants who reported smoking at least 1 cigarette per week
IQR: Interquartile range, 95% CI = 95% confidence interval

Table 2.7 Summary of participant level questionnaire data on demographics and characteristics, by exposure group

	Unexposed		Exposed		
Variable	**Summary Parameter**	**Range**	**Summary Parameter**	**Range**	**p-value**
n	105		74		
Age (years) (mean, 95% CI)	58.1 (55.6 - 60.6)	29 - 83	54.3 (51.1 - 57.6)	28 - 78	0.310
Education Category (% of population in each category: 1, 2, 3, & 4)[1]	1 – 4%, 2 – 37%, 3 – 19%, 4 – 40%		1 – 8%, 2 – 35%, 3 – 19%, 4 – 38%		0.844
Estimated H_2O Consumed (L / 2 days) (mean, 95% CI)	2.5 (2.2 - 2.8)	0 - 7.2	2.1 (1.8 - 2.5)	0 - 7.7	0.053
Rice Consumed (Y/N)	19%		11%		0.106
Estimated Rice Consumed (cups / 3 days) (mean, 95% CI)[2,3]	2.0 (1.0 - 3.0)	1 - 7	1.0 (1.0 - 1.0)	1 -2	1.000
Seafood Consumed (Y/N)	34%		42%		0.351
Estimated Seafood Consumed (ounce / 3 days) (mean, 95% CI)[2,3]	7.1 (5.0 - 9.0)	1 - 24	8.0 (6.0 - 10.0)	1 - 22	1.000
Chicken Consumed (Y/N)	51%		60%		0.247
Estimated Chicken Consumed (ounce / 3 days)(mean, 95% CI)[2,3]	5.1 (4.0 - 6.0)	1 - 16	5.6 (5.0 - 7.0)	1 - 16	0.526
Estimated As Consumed from Rice, Seafood and Chicken (μg) (median, IQR)[3]	9.3 (0 - 341.5)	0 - 2,020.8	12.8 (4.7 – 673.6)	0 – 5,151.6	0.357

[1]Education categories: 1 – primary, 2 – secondary, 3 – college, 4 – university
[2]Refers only to participants who consumed that product
[3]Rice [As] = 0.303 μg/g, chicken [As] = 0.0864 μg/g, seafood [As] = 2.36 μg/g
IQR: Interquartile range, 95% CI = 95% confidence interval

Table 2.7 Summary of participant level questionnaire data on demographic and characteristics, by exposure group (continued)

	Unexposed		Exposed		
Variable	**Summary Parameter**	**Range**	**Summary Parameter**	**Range**	**p-value[1]**
Estimated As Consumed from Water (µg / 2 days) (median, IQR)	**0.8 (0.2 - 2.4)**	0 - 47.2	**46.4 (24.7 - 89.3)**	0 - 1,798.8	**0.001**
Estimated Total As Consumed (µg / 3 days) (median, IQR)	**26.1** (3.5 - 371.9)	0 - 2,021.2	**151.1** (48.5 - 698.3)	0 – 5,260.7	0.192
Estimated Other Foods Consumed[2] (cups / 3 days) (median, IQR)	1.0 (0 - 1.0)	0 - 6	1.0 (0 - 2.0)%	0 - 12	0.771
Antacid (Y/N)	25%		24%		0.811
Antibiotics (Y/N)	1%		4%		0.183
Prescription Medication (Y/N)	71%		44%		0.541
Smoker (Y/N)	13%		18%		0.284
Estimated Smoking Amount (cigarettes/day) (mean, 95% CI)[3]	15.0 (10.0 - 20.0)	1 - 25	11.0 (6.0 - 15.0)	1 - 25	0.981
Estimated Bathing/Showering (times/week) (mean, 95% CI)	6.0 (6.0 - 6.0)	1 - 14	6.0 (6.0 - 7.0)	0 - 14	0.380
Use Shampoo with Selenium (Y/N)	5%		8%		0.402
Dye Hair (Y/N)	29%		36%		0.364

[1]Bolded data indicate variables which were significantly (p-value $\leq$ 0.05) different between categories
[2]Foods boiled in tap water or which use water to prepare (cooked vegetables, soups, pasta, etc.)
[3]Refers only to participants who reported smoking at least 1 cigarette per week
IQR: interquartile range, 95% CI = 95% confidence interval

Permutation analysis determined that participants from Hubbards had a significantly higher median age than those from Fall River ($p = 0.016$). Chi-square analysis revealed that a significantly larger proportion of participants from Fall River consumed seafood than did participants from Hubbards ($p = 0.041$), with 46% and 31% of participants reporting to have eaten seafood within the last 3 days, respectively (Table 2.6). However, for participants who reported eating seafood, the amount of consumption per person was not significantly different between locations.

The unexposed group was found to have consumed more water than the exposed group ($p = 0.040$) but had consumed significantly less As from drinking water when compared to the exposed category ($p < 0.001$). The unexposed population consumed an average of 0.8 μg of As from water, with an interquartile range (ICR) of 0.2 – 2.4 μg, while the exposed population consumed significantly more As from water, with an average of 46.0 μg consumed, and an IQR of 24.7 – 89.3 μg. While participants from Hubbards had a much higher maximum As consumption from water than those from Fall River (1,798.8 μg vs. 133.1 μg), only 5 participant from Hubbards had water As consumption values of over 200 μg over a 2-day period. Participants from Hubbards consumed 3.2 μg of As from water, on average, with an IQR of 0.4 – 52.1 μg, while participants from Fall River consumed an average of 4.0 μg of As, with an IQR of 0.3 – 33.7 μg, a non-significant difference. There were no other differences in participant level variables between exposure categories.

In Hubbards, the largest portion of estimated As consumed from food came from chicken (50%), while in Fall River, the largest portion came from seafood (49%). In both communities, seafood accounted for the highest percentage of As consumed from food sources when considering the total amount of As consumed from food and water combined (26% and 37% for Hubbards and Fall River, respectively).

2.4.3 Descriptive Statistics of Arsenic Concentrations in Biological Samples

Due to the short recruitment-to-visit period, some participants did not have adequate time to ensure their nails or hair had grown to sufficient lengths for collection. Therefore, not every participant had a full complement of nail and hair samples collected. Tables 2.8 (by location) and 2.9 (by water As exposure group) outline the number of collected samples and various sample descriptive statistics.

Table 2.8 Descriptive statistics of collected biological samples, by location and gender

Sample		n	[As] Mean, 95% CI	[As] Median	[As] Min - Max	Mass (mg) Median, SD
Fingernail	Total	147	0.4 (0.3 - 0.5)	0.2	0.077[1] - 4.1	20.6 (22.8)
	Hubbards Total	81	0.5 (0.3 - 0.6)	0.2	0.077[1] - 4.1	22.5 (24.2)
	Male	31	0.3 (0.2 - 0.4)	0.2	0.077[1] - 1.3	19.9 (27.8)
	Female	50	0.5 (0.3 - 0.8)	0.2	0.077[1] - 4.1	23.1 (21.9)
	Fall River Total	66	0.3 (0.2 - 0.4)	0.2	0.077[1] - 2.1	20.0 (21.1)
	Male	31	0.4 (0.2 - 0.6)	0.3	0.077[1] - 2.1	16.1 (21.0)
	Female	35	0.3 (0.2 - 0.4)	0.2	0.077[1] - 1.6	24.2 (21.2)
Toenail	Total	151	0.5 (0.3 - 0.7)	0.2	0.077[1] - 13.2	20.0 (31.0)
	Hubbards Total	87	0.4 (0.3 - 0.5)	0.2	0.077[1] - 2.9	25.3 (34.7)
	Male	36	0.5 (0.3 - 0.6)	0.2	0.077[1] - 2.8	17.3 (46.6)
	Female	51	0.4 (0.2 - 0.6)	0.2	0.077[1] - 2.9	25.5 (21.8)
	Fall River Total	64	0.6 (0.2 - 1.0)	0.2	0.077[1] - 13.2	18.9 (25.0)
	Male	31	0.3 (0.2 - 0.5)	0.2	0.077[1] - 2.4	20.1 (30.6)
	Female	33	0.9 (0.0 - 1.7)	0.3	0.077[1] - 13.2	18.2 (15.4)

[1]Data with an asterisk are equivalent to half of the minimum detection limit of ICP-MS for each particular biological sample
Minimum detection limits: fingernail – 0.152 μg/g, toenail – 0.152 μg/g
95% CI = 95% confidence interval, SD = standard deviation

Table 2.8 Descriptive statistics of collected biological samples, by location and gender (continued)

Sample		n	[As] Mean, 95% CI	[As] Median	[As] Min - Max	Mass (mg) Median, SD
Hair	Total	150	0.2 (0.1 - 0.2)	0.034[1]	0.034[1] - 2.3	66.3 (125.1)
	Hubbards Total	85	0.2 (0.1 - 0.3)	0.034[1]	0.034[1] - 2.3	72.3 (133.4)
	Male	30	0.3 (0.1 - 0.6)	0.034[1]	0.034[1] - 2.3	31.7 (57.5)
	Female	55	0.2 (0.1 - 0.2)	0.034[1]	0.034[1] - 1.6	90.3 (152.6)
	Fall River Total	65	0.1 (0.1 - 0.1)	0.034[1]	0.034[1] - 0.4	59.7 (113.5)
	Male	30	0.1 (0.0 - 0.1)	0.034[1]	0.034[1] - 0.4	38.9 (157.2)
	Female	35	0.1 (0.0 - 0.1)	0.034[1]	0.034[1] - 0.3	88.4 (53.7)
Urine	Total	172	55.8 (41.3 – 70.3)	26.0	0.355[1] - 884.2	
	Hubbards Total	103	48.9 (36.0 – 61.8)	23.9	0.355* - 364.3	
	Male	42	56.1 (34.6 – 77.6)	29.9	2.8 – 328.7	
	Female	61	44.0 (27.7 – 60.4)	22.5	0.355[1] - 364.3	
	Fall River Total	69	66.0 (35.0 – 97.1)	32.4	1.5 - 884.2	
	Male	34	84.9 (28.0 – 141.8)	31.6	4.6 – 884.2	
	Female	35	47.9 (19.2 – 76.1)	25.9	1.5 – 496.1	

[1]Data with an asterisk are equivalent to half of the minimum detection limit of ICP-MS for each biological sample
Minimum detection limits: hair – 0.068 μg/g, urine – 0.709 μg/L
95% CI = 95% confidence interval, SD = standard deviation

For each biological sample collected, there were participants with [As] above the recommended limits. Eleven fingernail and 14 toenail samples had an [As] of greater than or equal to the maximum recommended limit of 1 μg/g. Similarly, 4 participants had a hair [As] greater than the recommended limit of 1 μg/g. Twenty-five participants had total urinary [As] that exceeded the maximum recommended limit of 100 μg/L. All recommended maximum [As] were obtained from the Agency for Toxic Substances Disease Registry (ATSDR, 2007).

Permutation analysis found no significant differences between biological samples from the two locations or between genders, in total or within locations.

Table 2.9 Descriptive statistics of collected biological samples, by exposure group

Sample		n	[As] Mean, 95% CI	[As] Median[1]	[As] Min - Max	Mass (mg) Median, SD
Fingernail	Total	147	0.4 (0.3 - 0.5)	0.077[2]	0.077[2] - 4.1	20.6 (22.8)
	Unexposed	89	0.2 (0.2 - 0.3)	**0.077**[2]	0.077[2] - 3.6	20.6 (22.1)
	Exposed	58	0.7 (0.4 - 0.9)	**0.4**	0.077[2] - 4.1	21.1 (24.1)
Toenail	Total	151	0.5 (0.3 - 0.7)	0.077[2]	0.077[2] - 13.2	20.0 (31.0)
	Unexposed	87	0.3 (0.2 - 0.4)	**0.2**	0.077[2] - 3.2	22.2 (34.1)
	Exposed	64	0.8 (0.3 - 1.2)	**0.4**	0.077[2] - 13.2	16.0 (26.1)
Hair	Total	150	0.2 (0.1 - 0.2)	0.034[2]	0.034[2] - 2.3	66.3 (125.1)
	Unexposed	90	0.1 (0.1 - 0.2)	0.034[2]	0.034[2] - 2.2	59.6 (123.8)
	Exposed	60	0.2 (0.1 - 0.4)	0.034[2]	0.034[2] - 2.3	75.0 (128.1)
Urine	Total	172	56.0 (42.2 - 69.8)	24.9	0.355[2] - 661.1	
	Unexposed	100	44.8 (30.1 – 59.4)	**18.7**	0.355[2] – 447.5	
	Exposed	72	71.6 (45.6 – 97.6)	**34.5**	2.6 – 661.1	

[1]Bolded data denote significant differences ($p < 0.05$) between exposure categories
[2]Data with an asterisk were equivalent to half of the minimum detection limit of ICP-MS for each particular biological sample
Minimum detection limits: fingernail – 0.153 μg/g, toenail – 0.153 μg/g, hair – 0.068 μg/g, urine – 0.709 μg/L
95% CI = 95% confidence interval, SD = standard deviation

Fingernail, toenail, and total urine As median concentrations were found to be significantly higher in the exposed category versus the unexposed category ($p < 0.001$, $p < 0.001$, and $p = 0.007$, respectively). When considering only participants with water samples above the MDL, fingernail, toenail, and total urinary [As] remained significantly different between exposure groups ($p < 0.001$, $p = 0.001$, and $p = 0.007$, respectively), while differences in hair [As] between exposure groups remained non-significant. Additionally, due to varying urine concentrations across the sample population, descriptive statistics of SG-adjusted urinary [As] are also presented, in Table 2.10 by location and gender, and Table 2.11 by water As exposure group.

Table 2.10 Specific gravity adjusted total urinary arsenic concentration, by location and gender

Sample		n	[As] Mean (SD)	[As] Median[1]	[As] Min - Max
Urine	**Total**	172	55.8 (96.5)	26.0	0.355[2] - 884.2
	Hubbards Total	103	48.9 (66.0)	23.9	0.355[2] - 364.3
	Male	42	56.1 (69.1)	**29.9**	2.7 - 328.7
	Female	61	44.0 (63.9)	**22.5**	0.355[2] - 364.3
	Fall River Total	69	66.0 (129.2)	29.3	1.5 - 884.2
	Male	34	84.9 (163.0)	31.6	4.6 - 884.2
	Female	35	47.7 (82.9)	25.9	1.5 - 496.1

Data in μg/L
[1]Bolded data indicates significant differences in medians between genders
[2]Data with an asterisk are equivalent to half of the minimum detection limit of ICP-MS
SD = standard deviation

Table 2.11 Specific gravity adjusted total urinary arsenic concentration, by exposure group

Sample		n	[As] Mean (SD)	[As] Median[1]	[As] Min - Max
Urine	**Total**	172	55.8 (96.5)	26.0	0.355[2] – 884.2
	Unexposed	100	47.4 (96.5)	**22.5**	1.22 – 884.2
	Exposed	72	67.5 (95.1)	**37.1**	0.355[2] – 496.1

Data in μg/L
[1]Bolded data indicates significant differences in medians between exposure groups
[2]Data with an asterisk are equivalent to half of the minimum detection limit of ICP-MS
SD = standard deviation

In the SG-adjusted total urinary [As], a significant difference was found between males and females in Hubbards ($p = 0.0523$), with males having a higher median urinary [As] (Table 2.10). Median SG-adjusted total urinary [As] was significantly different between exposure categories ($p = 0.005$).

A Pearson correlation coefficient of 0.14 ($p=0.064$) was found between total SG-adjusted urinary [As] and water [As], while a coefficient of 0.64 ($p<0.001$) was found between SG-adjusted [TMA] and water [As]. Correlations between water [As] and fingernail, toenail, and hair [As] were 0.35 ($p<0.001$), 0.13 ($p=0.128$), and 0.59 ($p<0.001$), respectively.

2.4.4 Factors Associated with Arsenic Consumption

2.4.4.1 Arsenic Consumption from Water

The variables that were associated with the consumption of As from water (natural log transformed), based on linear regression analysis, are presented in Table 2.11. SG-adjusted urine [As] and well depth were not significant independent variables in the model as continuous variables, however they were both significant independent variables as categorical variables. Therefore, SG-adjusted urine [As] and well depth were included in the final model as categorical variables, which also improved the normality of residuals.

Table 2.12 Results of multivariable linear regression analyses of natural log-transformed arsenic consumption (μg) from water, with robust standard errors

Variable	Coef. (Robust St. Error)	95% CI	p-value
SG Adjusted Urine [As] Category			0.031
1 - <9 μg/L	Referent		
2 - 9 - 20 μg/L	1.19 (0.70)	-0.20 - 2.59	0.093
3 - 20 - 36 μg/L	1.52 (0.69)	0.15 - 2.90	0.031
4 - 36 - 72 μg/L	2.17 (0.72)	0.73 - 3.61	0.004
5 - >72 μg/L	2.18 (0.73)	0.73 - 3.63	0.004
Well Depth Category			0.031
1 - <23.0 m	Referent		
2 - 23.0 - 41.0 m	0.48 (0.71)	-0.94 - 1.90	0.503
3 - 42.1 - 55.0 m	1.68 (0.69)	0.30 - 3.07	0.018
4 - 55.1 - 87.0 m	0.33 (0.94)	-1.56 - 2.21	0.731
5 - >87.0 m	0.09 (0.80)	-1.50 - 1.68	0.910
Fingernail [As]	0.77 (0.29)	0.19 - 1.35	0.010
Water pH	1.53 (0.35)	0.84 - 2.23	<0.001
As Consumed from Rice	0.0005 (0.0001)	0.0003 - 0.0010	<0.001
Constant	-10.57 (1.93)	-14.41 to -6.73	<0.001
n	108		
Adjusted-R^2	0.5443		

Coefficients are in natural-log format
SG: specific gravity, 95% CI: 95% confidence interval
SG and well depth categories represent quintiles

- Water [As] intake model with back-transformed coefficients:

As consumption from water = 2.6×10^{-5} + [urine arsenic cat. 2*(3.3)] + [urine arsenic cat. 3*(4.6)] + [urine arsenic cat. 4*(8.8)] + [urine arsenic cat. 5*(8.9)] + [well depth cat. 2*(1.6)] + [well depth cat.3*(5.4)] + [well depth cat.4*(1.4)] + [well depth cat.5*(1.1)] + [fingernail arsenic concentration*(2.2)] + [water pH*4.6] + [As consumed from rice*1.0] + error

In comparison with the first category (lowest 20%), increasing categories of SG-adjusted urine [As] were associated with an increase in water As consumption of 3.3 (95% CI: 0.8 – 13.3), 4.6 (95% CI: 1.2 – 18.2), 8.8 (95% CI: 2.1 – 37.0), and 8.9 (95% CI: 2.1 – 37.7) times, respectively; all categories were significantly associated with increased water As consumption when compared to category 1. An increase in fingernail [As] from the 25th to the 75th percentile (0.077 – 0.4 μg/g) was associated with an estimated 1.3-fold (95% CI: 1.1 – 1.6) increase in As consumption from water. The depth of the well from which participants drew their drinking water, categorized into five quintiles, was associated with the amount of As consumed from water, however only one category was significant. In comparison to the baseline category of a well depth of less than 23.0m, participants whose well depth was between 42.1m and 55.0m (approximately 135 to 180 feet) were significantly associated with an increased estimated consumption of As from water, by 5.4 times (95% CI: 1.4 – 21.5). Additionally, an increase in water pH level from the 25th to the 75th percentile (5.5 – 6.6) was associated with an increase in estimated As consumption through water by 6.1 times (95% CI: 3.2 – 11.6). Finally, the amount of As consumed from rice, which includes dry-weight rice As (present in the grain itself) as well as As incorporated into the rice by absorption of water during the cooking process, was significantly associated with As consumed from water. An increase in rice As consumption from the 25th to the 75th percentile (0 – 412.7 μg) was associated with an estimated increase in As consumed from water of 1.2 times (95% CI: 1.0 – 1.5).

SG-adjusted urinary [As] was not significantly associated with water As consumption as a continuous variable in any linear regression analyses. No other demographic information, such as age, gender or location, was significantly associated with As consumption from water. The adjusted R^2 for this linear regression was 0.5443 with 108 observations due to missing data for some observations, such as urine and fingernail samples, well depth, or water pH. The final model had homoscedastic residuals that were normally distributed with no significant outliers.

2.4.4.2 Arsenic Consumption – From Food, & From Food and Water Combined

Logistic regression analyses were similar between food As consumption and total As consumption. For this reason, both logistic regression analyses will be considered together. The variables that were most associated with As consumption from food and total As consumption, based on logistic regression analysis, are presented in Table 2.13. Again, model residuals were skewed when SG-adjusted urine [As] and well depth were in the model as continuous variables, therefore they were categorized, leading to a better goodness of fit. Income was a categorical variable from the beginning.

Table 2.13 Results of multivariable logistic regression analyses, with robust standard errors, of arsenic consumed from food and total arsenic consumed from food and water

Variable	Food As Ingestion Model			Food and Water As Ingestion Model		
	OR (Robust St. Error)	95% CI	p-value	OR (Robust St. Error)	95% CI	p-value
SG Adjusted Urine [As] Category			<0.001[1]			0.002[1]
1 - <9 μg/L	Referent			Referent		
2 - 9 - 20 μg/L	2.23 (1.37)	0.67 - 7.42	0.192	1.21 (0.79)	0.34 - 4.32	0.767
3 - 20 - 36 μg/L	1.60 (0.95)	0.50 - 5.12	0.430	1.51 (0.92)	0.46 - 4.97	0.497
4 - 36 - 72 μg/L	8.04 (5.06)	2.34 - 27.62	0.001	5.82 (4.19)	1.42 - 23.85	0.014
5 - >72 μg/L	9.37 (5.84)	2.76 - 31.78	<0.001	10.26 (6.75)	2.82 - 37.27	<0.001
Income Category			0.097[1]			-
1 - <$30,000/year	Referent			-		
2 - $30,000 – $50,000/year	6.01 (5.82)	0.90 - 40.12	0.064	-	-	-
3 - >$50,000/year	6.60 (5.77)	1.19 - 36.59	0.031	-	-	-
Well Depth Category			-			0.082[1]
1 - <23.0 m	-			Referent		
2 - 23.0 - 41.0 m	-	-	-	8.83 (7.03)	1.85 - 42.08	0.006
3 - 42.1 - 55.0 m	-	-	-	2.74 (1.81)	0.75 - 9.99	0.126
4 - 55.1 - 87.0 m	-	-	-	1.45 (1.03)	0.36 - 5.82	0.604
5 - >87.0 m	-	-	-	1.90 (1.28)	0.51 - 7.12	0.340
Constant	0.05 (0.06)	0.01 - 0.39	0.004	0.17 (0.11)	0.05 - 0.57	0.004
n	156			157		
Pseudo-R^2	0.1526			0.1865		

Table 2.13 Results of multivariable logistic regression analyses, with robust standard errors, of arsenic consumed from food and total arsenic consumed (continued)

[1]Overall p-value for categorical variable
OR: odds ratio, SG: specific gravity
SG and well depth categories represent quartiles and quintiles, respectfully

- Food As intake model (expressed as coefficients): ln[p1 / 1 – p1] = -2.9 + [urine arsenic cat. 2*(0.8)] + [urine arsenic cat. 3*(0.5)] + [urine arsenic cat. 4*(2.1)] + [urine arsenic cat. 5*(2.3)] + [income cat. 2*(1.8)] + [income cat. 3*(1.9)]

- Total As intake model (expressed as coefficients): ln[p2 / 1 – p2] = -1.8 + [urine arsenic cat. 2*(2.2)] + [urine arsenic cat. 3*(0.4)] + [urine arsenic cat. 4*(1.8)] + [urine arsenic cat. 5*(2.3)] + [well cat. 2*(2.2)] + [well cat. 3*(1.0)] + [well cat. 4*(0.4)] + [well cat. 5*(0.6)]

p1 = probability of food As intake > 9.3 μg; p2 = probability of total As intake > 86.4 μg

For each analysis, increasing SG-adjusted urinary [As] categories were associated with increased odds of being in the high As consumption category. Participants in the 60% - 80% and the >80% range of SG-adjusted urinary [As] were more likely to be in the high consumption category, by 8.0 (95% CI: 2.3 – 27.6) and 9.4 times (95% CI: 2.8 – 31.8), respectively, as compared to the lowest 20% of SG-adjusted urinary [As] values. Other SG-adjusted urinary [As] categories were found not to be significantly different from the lowest 20%. For the estimated total As consumed model, a similar trend was seen, where participants in the 60 – 80% and >80% SG-adjusted urinary [As] categories were associated with an increased odds, by 5.8 (95% CI: 1.4 – 23.9) and 10.3 times (95% CI: 2.8 – 37.3), respectively, of being in the high total As consumption category, as compared to participants in the lowest 20%.

Household income category was significantly associated with the estimated As consumption from food, but not of total As consumption. Participants in the middle-income category and highest income category were 6.0 (95% CI: 0.9 – 40.1) and 6.6 times (95% CI: 1.2 – 36.6) more likely, respectively, to be in the high estimated food As consumption group than those in the lowest income group. The depth of the well from which a participant obtained their drinking water was significantly associated with total As consumption, but was not associated with As consumption from food. In this analysis, participants' wells that were 23.0 – 41.0 m deep had significantly higher odds, by 8.8 times (95% CI: 1.9 – 42.1), to be in the high estimated total As consumption group. All other well depth categories were found to not be at significantly different odds than the first category.

2.5 Discussion

Our results showed that SG-adjusted urine [As] was the only biomarker significantly associated with food As consumption and with food and water As consumption combined. This finding is not surprising considering that the diet of the participants did contain substantial amounts of seafood, rice and chicken, three foods reported to contain some As (Dabeka et al., 1993; Schoof et al., 1999).

Our study also found that both SG-adjusted urine [As] and fingernail [As] were significantly associated with water As consumption, however the model (Table 2.12) did not allow for direct comparisons between the two variables to see which was more significantly associated with water As consumption because SG-adjusted urine [As] is a categorical variable and on a different scale from the continuous fingernail [As] variable. However, when each variable was removed from the final model, decreases in the adjusted-R^2 value of 0.0896 and 0.1295 were observed for urine [As] and fingernail [As], respectively, indicating that fingernail [As] explains a slightly larger proportion of the total variation in water As consumption than urinary [As]. This change in adjusted-R^2 value is akin to assessing which variable has a greater impact on the model and of the statistical significance of each. In contrast to the difference in adjusted-R^2 values, however, an increase from 25% - 75% (the interquartile range) in fingernail [As] (from 0.077 – 0.4 μg/g) was associated with an increase of 1.3-times the estimated amount of As consumed from water. Conversely, an increase from the 20-40% urinary [As] category to the 60-80% category (roughly analogous to the interquartile range) was associated with an increase in water As consumption of 2.7–times

(exponentiation of the difference in quintile coefficients), over double that found for fingernail [As], suggesting that urinary [As] is more highly associated with water As consumption than fingernail [As]. This IQR-related analysis is not associated with statistical significance of the independent variables, and relates only the magnitude of change associated with those changes in IQR-related differences. However, the fingernail [As] IQR and the difference between SG-adjusted urinary [As] from the 2nd to the 4th quintile are not equal. An increase in fingernail [As] from the 25th to the 75th percentile is an increase of 50%, while in comparison, an increase from the 2nd to the 4th quintile groups of the SG-adjusted urinary [As] variable represents the average change for all observations falling within those quintile categories. However, comparing interquartile-associated differences with 2nd to 4th quintile differences are not equal, because these quintile categories extend beyond the 25th and 75th percentiles, and the coefficients for the quintiles represent the average change in water As consumption for all observations falling within the quintile category. Since we were interested in knowing whether one biomarker was better than other biomarkers for assessing 3 day As exposure, the amount of variation accounted for is preferred, and therefore fingernails may be slightly better than urine in this regard. However, it remains unclear whether urine or fingernail [As] is truly the better biomarker for water As consumption.

The average total urinary As concentrations found in our study population (Table 2.10) were 2 – 3 times higher than those found in a study by Health Canada of 5,000 Canadian residents exposed to normal background levels of As (Health

Canada, 2010). A report using National Health and Nutrition Examination Survey (NHANES) data on the background levels of As in an American population also found average total urinary As concentrations of up to four times lower than in the present study (Caldwell et al., 2009). However, our study was not designed with the goal of determining the As exposure of a representative population because we were more interested in obtaining a broad spectrum of exposures to better understand the methods and protocols for As biomonitoring. This broad range of As exposures was an advantage in our study because it provides an enhanced sample size and therefore representativeness of the identified associations at high and moderate exposures, not just low exposures.

Some studies have used urinary [As], fingernail or toenail [As], and hair [As], as a surrogate for the degree of exposure an individual has had to As. Although hair and nails are known to represent exposure over a longer time frame than urinary [As], they are often applied in studies that relate to short-term, or unknown, As exposure information, or rely only on the concentration of As in water (Button, Jenkin, Harrington, & Watts, 2009; Gault et al., 2008; Hinwood et al., 2003; Karagas et al., 1996).

Correlations between total urinary [As] and water [As] have been reported in ranges from 0.71 to 0.96 (Y. Chen et al., 2010; Concha, Nermell, & Vahter, 2006; B. K. Mandal, Ogra, Anzai, & Suzuki, 2004). While the correlation between water [As] and total SG-adjusted urinary [As] in the present study was just 0.14, the correlation between TMA and water [As] was much higher, at 0.64, likely because food As ingestion was contributing substantially to the urinary [As]. Previous

studies have also found significant correlations between fingernail, toenail, and hair [As] and water [As] ranging from 0.70 – 0.74, 0.42 – 0.67, 0.45 – 0.86, respectively (Y. Chen et al., 2010; Concha et al., 2006; Gault et al., 2008; Karagas et al., 1996, 2000; B. K. Mandal et al., 2004; Rivera-Nunez, Meliker, Meeker, Slotnick, & Nriagu, 2011). However, in general, the correlations between these biomarkers and water [As] were lower in the present study than in other published literature. While factors such as age, sex, race, genetics, and geographical location, have all been found to have at least some impact on the relationship between biomarkers of As exposure and water [As] or water As consumption (Caldwell et al., 2009; Hossain et al., 2012; Lindberg et al., 2008; Rahman et al., 2006; Slotnick, Meliker, AvRuskin, Ghosh, & Nriagu, 2007; Vahter, 2000; Vahter et al., 1995), it is unclear as to why the correlation between these biomarkers of As exposure and water [As] are lower in this study than in others. Factors such as age, gender, or location of residence were not significant factors in any of the performed regression analyses. Participants could have had exposures to As that were not accounted for, such as inhaled aerosols or skin contact. Previous research has shown that hair samples can be highly correlated with long-term As ingestion (from water and food) as well as estimated lifetime As exposure (Gault et al., 2008; Mosaferi et al., 2005), but also that hair [As] can vary greatly, even under similar circumstances (Hindmarsh et al., 2002; Hindmarsh, McLetchie & Heffernan, 1977). Similarly, fingernail and toenail samples have been shown to be highly correlated with ingested As and long-term exposure (Gault et al., 2008; Kile et al., 2007).

Urinary [As] was expected to correlate highly with ingested As within the last 2 and 3 days because ingested As is quickly metabolized and excreted in the urine, with 50% of the ingested As being excreted within 28 hours (Buchet, Lauwerys, & Roels, 1981). While nail and hair [As] were expected to correlate with As ingestion, it was expected that they would be less correlated than urine [As], because they are more representative of exposure over longer time periods. Arsenic is deposited in nails and hair more slowly and over a longer period of time than it is excreted in urine (Maes & Pate, 1977; Shapiro, 1967; R. A. Smith, 1976; S. Smith & Hendry, 1934). Although fingernail [As] was identified as significantly associated with As consumption from water over a 2 day period, fingernail samples may be better suited for assessing long-term As exposure rather than relatively short-term exposure, as examined in this study. A stable water [As] and water consumption patterns could explain why fingernails were significant in the final model, even while controlling for urine As. Some studies use hair and nail [As] as a surrogate of previous As exposure, in an attempt to predict risk of disease (Amaral et al., 2012; Heck et al., 2009; Kwong et al., 2010; Paul et al., 2011), however our study shows that hair and toenail [As] may not be as representative of previous exposure as commonly assumed.

It was expected that no biological sample, aside from urine, would be significantly associated with food As consumption. Although inorganic As ingested through food will be deposited in nails and hair in small amounts, the majority of consumed food As came from seafood in larger organic molecules, which have not been shown to accumulate in these tissues (B. K. Mandal et al., 2004; Badal Kumar

Mandal, Ogra, & Suzuki, 2003). The questionnaire used to estimate the amount of As each person ingested from different sources was only a snapshot in time of a person's normal eating and drinking habits. Information on water consumption was collected for the previous two days, and the previous three days for food consumption. Over such a short time period, it is difficult to assess the average or "normal" As ingestion rate over a longer period of time.

While fingernail [As] was significantly associated with As consumption from water (Table 2.12), neither hair nor toenail [As] was significant in any analyses, for a number of reasons. Hair sample size is most often larger than nail samples, in total length (not mass), which represents a longer time periods of exposure. Also, although standard procedures for hair collection have been proposed, many studies do not follow suggested guidelines, as is evident from different sample collection strategies in published literature (Gault et al., 2008; Hindmarsh, 2002; Mosaferi et al., 2005; Muikku, Puhakainen, Heikkinen, et al., 2009); collected samples were not uniform in length, thereby representing different time periods of exposure. Additionally, previous research has shown that hair, and to a lesser extent nails, can be contaminated by external sources of As, causing (Frisch & Schwartz, 2002; Hindmarsh, 2002). Urine does not suffer from this type of contamination.

The amount of As consumed from eating cooked rice, which includes As present in the raw rice grain as well as As absorbed from the water in which it was boiled, was a significant positive predictor of As consumed from water (Table 2.12). This finding is logical since the largest portion the total As consumed from

the rice comes from the water in which it was boiled. In comparison to the water it is boiled in, the amount of As found naturally in rice is very little. In essence, the significance of the rice As consumption variable is a reflection of the amount of water that is consumed via the rice. While the concentration of As in the rice that was consumed by each participant was assumed to be 0.3 μg of As per gram of uncooked rice, based on work by Schoof et al. (Schoof et al., 1999), the actual concentration of the rice that was consumed by participants is not known. In areas where the concentration of As in rice consumed by locals is known, this staple food has been shown to be a significant source of As in the diet and is associated with increased urinary [As] (Cascio et al., 2010; Jackson, Taylor, Karagas, Punshon, & Cottingham, 2012; Sanz, Muñoz-Olivas, Cámara, Sengupta, & Ahamed, 2007).

Food As intake estimates that were included in logistic regression models (Section 2.4.4.2) represented the amount of a particular food item consumed (i.e. in grams) which was scaled equally for all participants by the estimated As concentration for each food item. Therefore, while the coefficient values in logistic regression models may be rough estimates of the association between dependent and independent variables, associated p-values remain as representations of the association between dependent and independent variables and are unaffected by the assumed concentration of As assigned to each item. While obtaining actual As content information for all consumed foods would be ideal, this would represent a major time and cost increase that was not feasible for this study. The combination of food questionnaire data and estimates of As in foods is a common method of obtaining an estimate of individuals' exposures to As via food (Caldwell et al., 2009;

MacIntosh et al., 1997; Xue, Zartarian, Wang, Liu, & Georgopoulos, 2010).

Water consumption was estimated over a two-day period to get a representation of a persons' drinking habits over that period. Due to seasonal variations, activity levels, etc., the estimated amount of water consumed by individuals may not accurately reflect a person's "usual" drinking habits (Ryan, Huet, & MacIntosh, 2000). Seasonal variations in water consumption have been found to fluctuate between summer and winter months, with intake estimates varying by as much as 27% in India (Hossain et al., 2012), to between 6 – 30% in the United States (Michaud et al., 1999; Ryan et al., 2000). While this is a limitation of short-duration studies, it is not uncommon for this type of estimation to be used in studies using the [As] of a biological sample as a surrogate for As exposure through water.

Food consumption was estimated over a three-day period because recall of food consumption over a longer period was unrealistic. As with water As intake estimates, food intake estimates were expected to be representative of the time-frame covered, though they may not be accurate representations of a person's eating habits over a longer time period of weeks to months, which may also depend upon seasonal variability.

Well depth was found to be significantly associated with water As consumption and total As consumption (Table 2.12 and Table 2.13), but not in regression analysis for food As consumption. Only the second and third well depth categories (23.0 – 55.0 m) were significantly associated with higher As consumption, indicating that water at these depths may have an increased As

concentration. It would be expected that well depth would have no effect on As consumption from food, but could possibly play a role in As consumption from water if As were more common in certain depths of the soil (between 23.0 – 55.0 m) in this area. While this trend of increasing water [As] with increasing well depth has been reported elsewhere (Becker, Smith, Greer, et al., 2010; Chowdhury et al., 1999; Kim, Houseman, Breton, et al., 2011; McCurdy, 1980), evidence of the opposite association (Kim, Nriagu & Haack, 2003) has been observed. Chowdhury et al. (1999) reported that the distribution of naturally occurring As in earth's crust increased to a depth of 22m and decreased afterwards (Chowdhury et al., 1999). The effect of soil/rock composition and well depth have not been extensively studied in Nova Scotia, and our findings would benefit from further study to elucidate what effect these factors have on water [As] in these specific areas. If future studies discover that certain levels of soil/rock are more likely to have higher levels of As in certain areas, then advisories could be given to people building new homes, or renovating older wells, in those areas.

The result that income category is a significant predictor of As exposure in food consumption but not water or total As consumption (Table 2.13), can be explained by seafood consumption. With each increase in income category, more participants reported consuming seafood in the last 3 days, and for participants who consumed seafood, the amount of seafood consumed increased with increasing income level. Even in these communities in eastern Canada that are not far from the Atlantic Ocean, seafood can be an expensive commodity, and its consumption may therefore be limited in households whose budget is low. As

discussed in the results section, a major portion of As ingested from food, and therefore also total As ingested, came from seafood (a more in depth discussion on seafood As consumption, and its relation to urinary As concentrations can be found in Chapter 3). Previous studies corroborate the finding that seafood consumption is associated with the total amount of As consumed (Navas-Acien, Francesconi, Silbergeld, & Guallar, 2011), which emphasizes the impact that income may have on total As consumption in these communities.

In both linear and logistic regression analyses, potential explanatory variables related to selenium (selenium concentration in water, urine, hair, and nails) were investigated as potential confounders, due to the known biological interactions between As and selenium (Gailer, 2007). No confounding effects of selenium were observed in any regression models, and therefore these potential confounding variables were not included in final models. Due to the sampling method used, there existed the possibility of observing effects of clustering at the household and location levels. Participants who lived in the same household or location were potentially more similar to one another (consumption and lifestyle habits, genetic traits, etc.) than participants who lived in different houses (average of only 1.6 persons per household) or locations, and this potential clustering effect should be considered in the statistical analyses. During the model-building process for all linear and logistic models presented above, models were constructed using random effects variables that accounted for clustering at the household and location levels. In all of these models, the random effects portion of the variance observed in the outcome variables contributed little to the total observed variation.

Because models including a random effects variable did not significantly contribute any information that was not found in models without random effects variables (i.e. changes in coefficient values of <25%), the simpler models with only fixed effect variables were presented and discussed. However, these fixed effects models were fitted using robust standard errors, to control for the household effect .

A couple of information biases should be noted. Due to time constraints during field data collection, many participants had biological samples collected less than 48-hours after they were asked to participate in the study and because of this, some nail samples were of insufficient mass to be analyzed for the presence of As. As well, participants with little advanced warning of sample collection were often unable to reliably recall what they had eaten or drank in the last 3 days, whereas participants with ample warning were able to make mental or physical notes on what they had eaten. This may have led to an underestimation of water consumed during the previous 2 days or specific foods that were eaten over the last 3 days, which would influence the association between the biomarkers and what was reported as As consumption. Specifically, data quality on As consumption from seafood may benefit from altered data collection methods in future studies. Due to the potential recall bias when participants were asked about the amount and type of seafood consumed, individual values for [As] for individual types of seafood were not feasible. In this study, a single value for As content in seafood was used regardless of the actual seafood type, which is known to vary between species.

A couple of selection biases should also be noted. To collect as much information as possible from individuals whose water sample contained greater

than 10 μg/L of As, each household in this category was contacted and asked to participate. In contrast, only a fraction of participants in the <10 μg/L of As category were contacted, albeit by random selection. This technique of participant selection may have introduced some selection bias into the study. As well, when individuals were contacted to take part in the study, they were informed of their water [As]. For participants whose water was above the MCL of 10 μg/L, an adjustment of normal drinking habits may have occurred in the time between recruitment and sample collection. This could misrepresent the amount of water consumed on a daily basis before the participant was aware of their water [As], and therefore might not accurately reflect past exposure. This may be especially important when comparing long-term biomarkers such as hair and nails to the amount of As consumed from water in the last 2 days, which was taken to be representative of "normal" exposure for each individual.

2.6 Conclusion

SG-adjusted urinary [As] was consistently positively associated with estimated acute As exposure levels from water, food, and water and food combined. Fingernail [As] and water pH were also significantly positively associated with water As consumption while income was significantly associated with high food As consumption. The depth of the well being used to provide drinking water was also significantly associated with the amount of As consumed from water, and the total amount of As consumed, particularly between 23 and 41 meters, suggesting that some layers of soil or rock may contain more As than others. Detailed knowledge of the geology and its relationship to As concentration

could be a useful tool in determining which areas may be at risk of elevated levels of As in groundwater. Neither toenail nor hair [As] was significantly associated with As consumed from water, food, or both, and therefore should not be utilized for determining short-term As exposure.

For future studies that involve a questionnaire to collect information on seafood consumption (or for any other food or drink), it is recommended that an appropriate length of time between recruitment and sampling be used (several weeks). Not only does this provide time for participants to allow their fingernails, toenails and hair to grow to an appropriate length for sampling, it also allows participants to keep a daily log of food and drink consumption log.

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3 Factors Associated with Urinary Arsenic Metabolites

3.1 Abstract

A useful biomarker of arsenic (As) exposure can be the concentration of As ([As]), both total and speciated, in urine. This study focused on which demographic, lifestyle and consumption habits were associated with different urinary As metabolites.

Based on a screening survey, 179 individuals comprised the sample group, coming from 108 unique households from two locations (107 individuals from 67 homes in Hubbards, 72 individuals from 41 homes in Fall River). Participants were chosen to maximize the number of people whose main water source contained a water [As] of at least 10 μg/L, the current maximum contaminant limit for As in drinking water. Participants were visited at their home for a face-to-face interview to collect water and food consumption information, as well as demographic and lifestyle factor information. At this time, a water sample and a spot urine sample were collected. Both water and urine were sent to Acadia University in Wolfville, NS, for quantification of As, total and speciated concentrations, by inductively coupled plasma-mass spectrometry and high performance liquid chromatography, respectively.

Using multiple linear and logistic regression analysis, it was found that the [As] of a person's main water source was a consistent and significant direct predictor of all urinary As metabolite concentrations, total and speciated, with the exception of arsenobetaine, which is an organic form of As obtained mainly from seafood

consumption. Additionally, seafood consumption was found to be a significant predictor of many urinary As metabolites, including highly toxic arsenite, suggesting that seafood-derived As compounds (arsenosugars, arsenolipids, arsenocholine) may be metabolized in the body, supporting recent research in this field. To a lesser degree, age of a participant, and pH level of the main water source were significant positive predictors of various urinary As metabolites.

3.2 Introduction

Arsenic can be found in many different chemical arrangements (known as species) and is most often found in water in the inorganic trivalent (arsenous acid, H_3AsO_3) and pentavalent forms (H_2AsO_4 and $HAsO_4^-$) (Agency for Toxic Substances and Disease Registry (ATSDR), 2007). Although few studies have been performed to investigate the health effects of individual As species on large scale populations, there have been many epidemiological investigations into the health effects of As, resulting in clear evidence that chronic exposure to high concentrations of As can lead to a greater risk of different types of cancers, including: skin, kidney, urinary bladder, and lung. (Banerjee et al., 2008; Chiou et al., 2001; Ferreccio et al., 2000; Guo, Chiang, Hu, et al., 1997; Kurttio, Pukkala, Kahelin, et al., 1999; Yu, Liao, & Chai, 2006). In Canada, the province of Nova Scotia was reported by the Canadian Cancer Society to have the highest age-standardized incidence rates of cancer for males for the last 5 years, and the last 3 years for females (Canadian Cancer Society, 2010). In particular, Nova Scotia residents suffer from the highest rates of bladder, kidney and lung cancer in the country. Groundwater surveys have also found

concentrations of As of concern in many areas of Nova Scotia (Gibbons & Gagnon, 2010; Meranger, Subramanian, McCurdy, 1984).

Although a maximum [As] of 10 μg/L in water is recommended (World Health Organization (WHO), 2008), many areas of the world have neither the knowledge nor resources to assess the quality of their drinking water. Therefore, additional research into As biomonitoring is necessary. Assessing the degree to which people have been exposed to As can be achieved in several ways. The most obvious and common method is to analyze a person's household drinking water for the [As], which can be accomplished by use of an inductively coupled plasma mass spectrometer (United States Environmental Protection Agency, 1994) (ICP-MS). After determining the [As] in a person's water supply, investigation into the amount of this water (and other liquids mixed with this water, or foods cooked or processed with this water) that is consumed will estimate an average daily consumption of As from a personal water source. However, this method is not able to capture any water exposure to As that may occur outside of the home, unless other water sources outside the home are tested for [As] as well. Arsenic consumed in foods will also not be included in this As exposure estimate, nor will any dermal or aerosol exposure. Similar estimates of [As] in food, and volumes consumed, could also be determined, capturing food exposure to As, but not dermal or aerosol exposure. Therefore, assessing an As biomarker may be more representative of total As exposure.

Urine As is one possible biomarker (Hays, Aylward, Gagné, Nong, & Krishnan, 2010; Hsueh et al., 2002; Hughes, 2006; Marchiset-Ferlay, Savanovitch, & Sauvant-Rochat, 2012; Orloff, Mistry, & Metcalf, 2009). Once ingested and metabolized by the body, most As is rapidly excreted in the urine. In a human experiment, it was found that after a single 500 μg dose of sodium arsenite approximately half of the As excretion in urine over a 4-day period occurred in the first 28 hours. After this 28-hour mark, urinary [As] steadily tapered off (Buchet, Lauwerys, & Roels, 1981a). Therefore, testing urine for concentrations of total As and its species could be a method of determining As exposure – particularly recent As exposure.

Using ICP-MS, the concentration of total As can be determined not only in water, but also in urine samples. Additionally, with the coupling of ICP-MS with High Performance Liquid Chromatography (HPLC), the concentration of individual As species from urine and water samples can be identified. When As is ingested through a water or food source, the body is able to metabolize the inorganic As, through a series of reduction and methylation steps, into an organic form. Pentavalent As (arsenate, As(V)) is reduced to trivalent As (arsenite, As(III)), which is then methylated to form monomethylarsonic acid (MMA(V)). Subsequently, MMA(V) is reduced to form monomethylarsonous acid (MMA(III)), which is methylated to dimethylarsinic acid (DMA(V)). Finally, DMA(V) is further reduced to produce dimethylarsinous acid (DMA(III)) (Cullen, McBride, Reglinski, 1984).

The inter-individual ability to methylate and reduce As is quite large, and is dependent upon genetics, age, diet, and other factors (Loffredo, Aposhian, Cebrian, et al. 2003). Likewise, the concentration and type of As found in urine depends highly on the form of As ingested (As(III), As(V), AsB, etc.) and specific gravity of the urine. The average urine As profile, regardless of inter-individual variation, specific gravity and ingestion differences, is approximately as follows: 10-30% inorganic As, 10-20% MMA, and 60-70% DMA (Vahter, 1999). In the past, the prevailing theory was that As methylation was a mechanism of detoxification. However, in the last decade, it has been recognized that methylation of As to MMA(V) and DMA(V), which are less acutely toxic than inorganic As(III), can produce reactive intermediates, MMA(III) and DMA(III), which are now considered genotoxic (Kitchin, 2001; Kligerman et al., 2003; Mass et al., 2001; Thomas, Styblo, & Lin, 2001). Due to the wide range of urinary As metabolites, and the varying degree of toxicity between them, it is important to take each As species into consideration when using [As] in urine to predict As exposure.

Due to its ability to create strong bonds with sulfhydryl groups, As binds preferentially with keratin and therefore, As is also excreted in small amounts in hair and nails (Hopps, 1977). As a result, the testing of hair and nails for their [As] is another method of determining As exposure – although with the slow growth of hair and nails, this method is considered more appropriate for estimating long-term As exposure (Orloff, Mistry, Metcalf., 2009).

In a previous study (see chapter 2), it was found that total urinary [As] was the most consistent and noteworthy predictor of estimated As ingested from water and food, not hair or nails. The emphasis of that study focused on total As in urine and did not examine speciated forms. While many studies have investigated the role of total urinary As, as well as individual As species, on health outcomes or other endpoints including genetic damage, As related cancers, neuropathy, lowered cognitive development, hyperpigmentation, keratosis, skin lesions and others, (Ahsan et al., 2006; Asadullah, 2008; Basu et al., 2004; Buchet et al., 1981a; Buchet, Lauwerys, Roels, 1981b; Caldwell et al., 2009; Chen et al., 2009; Kile et al., 2011; Kitchin, 2001), few studies have investigated what factors are associated with elevated or decreased concentrations of individual As species in urine. To better understand potential health risks of As species, it is important to elucidate which demographic, lifestyle and consumption factors are most associated with elevated urinary [As], total and speciated.

The objective of this study was to determine which factors most predict the concentrations of total urinary As, urinary arsenite (As(III)), urinary arsenate (As(V)), urinary monomethylarsonic acid (MMA(V)), urinary dimethylarsinic acid (DMA(V)), urinary arsenobetaine (AsB), and total urinary As minus arsenobetaine (TMA).

3.3 Materials and Methods

3.3.1 Screening Procedure to Identify a Sampling Frame and Study Groups

See section 2.3.1 of this thesis for methods pertaining to the identification and selection of the sampling frame and study groups, and collection of demographic and other participant information.

3.3.2 Sample Collection and Laboratory Analysis

In July of 2010, during a face-to-face interview, urine was collected in a 100ml trace-metal-free bottle (Fisher Scientific). For urine collection, participants were instructed to initially void a small amount of urine into the toilet before beginning their sample collection in the bottle, if possible. Additionally, a second water sample was collected during the home visit, to obtain the most accurate reflection of water [As] over the last 3 days. After collection and pre-processing, urine and water samples were sent to Acadia University in Wolfville, Nova Scotia. All samples were analyzed for total [As] by inductively coupled plasma mass spectrometry (ICP-MS) and were also analyzed using high performance liquid chromatography in conjunction with ICP-MS (HPLC-ICP-MS) to determine the concentration of the different As species present in each sample. The minimum detection limit for all [As] in water was 0.017 μg/L, and 0.709 μg/L for As in urine.

3.3.3 Descriptive Statistical Analyses

For the purpose of comparing groups for statistical analyses, participants were broken into two separate categories based on their degree of As exposure. Participants whose water contained less than 10μg/L were placed in the

"unexposed" category, and all others, with water [As] of ≥ 10 μg/L, were placed in the "exposed" category.

Due to variations in urine sample concentration (very dilute or concentrated samples), urinary [As] (total and speciated) were adjusted to better represent its true value by correcting for the specific gravity (SG) of the sample. Specific gravity of urine samples was obtained by determining its refractive index, using a PAL-10S Portable Digital Urine Specific Gravity Refractometer (ATAGO Ltd.), and each sample was adjusted by using the following formula:

$$Specific\ Gravity - Adjusted\ Urinary\ \ As\ \ \frac{ug}{L}\ =$$

$$Urine\ \ As\ \ \frac{ug}{L}\ \ * [\frac{mean\ SG-1}{sample\ SG-1}]$$

Adjusting the urinary [As] in this way proportionately increased the [As] for samples that were more dilute than average, and decreased the [As] for samples that were more concentrated than average.

To investigate differences in urine As metabolites between location, gender or exposure groups, permutation analysis was performed (Baglivo, 2005). Permutation analyses were used to investigate statistically significant differences between group medians, by running 10,000 iterations of re-sampling with replacement, and comparing the true group median with that of the permutation median. This is a preferred method of determining statistically significant group differences in non-normally distributed data as a preliminary step prior to multivariable modeling.

3.3.4 Analytical Statistical Analyses

Relationships between urinary [As] and demographic, consumption, and other questionnaire information were investigated through multivariable linear regression analysis, using total urine [As], or one of the As species, as the outcome variable. Due to a number of urine samples with concentrations of As metabolites of less than the minimum detection limit of the ICP-MS (see section 2.3.3), linear regression analyses were performed on all available non-zero concentrations. The number of samples with As metabolites of <MDL, out of a total of 172 urine samples, were: total As – 1, As(III) - 110, As(V) - 121, MMA(V) - 77, DMA(V) - 21, AsB – 34, and total As minus AsB (TMA) – 22. In this way, regression analyses reflected which predictors were associated with urine As metabolites only when they were greater than 0.709 μg/L. As previously discussed in Section 2.3.4 of this thesis, in these linear regression models, the outcome variables were natural-log transformed to obtain a normal distribution of values and model residuals. Coefficients of continuous independent variables in log-transformed linear regression models will be back-transformed and interpreted as the percent change in the outcome variable that occurs when increasing the continuous independent variable value from the 25^{th} percentile to the 75^{th} percentile (i.e. the interquartile range). For categorical variables, all percent changes in the outcome variable will be interpreted with respect to the baseline category of the independent categorical variable.

To account for any biases that may be produced by only running linear regression analyses on only those samples with an As metabolite concentration of greater than the MDL, logistic regression analyses were also performed for

metabolites that had less than 80% of total available samples represented in linear regression analyses (As(III), As(V), and MMA(V)). Logistic regression analyses were performed for urinary As(III), As(V), and MMA(V), with a cut-off value of the MDL (0.709 μg/L) used to classify a sample as positive or negative. Analyses that did not contain a sample size of 172 were due to missing data in the predictor variables, and not the urinary As metabolite. Because >80% of all samples were above MDLs for total As, DMA, AsB, and TMA concentrations, there are too few "zero values" to adequately perform logistic regression for those metabolites.

Whenever possible, predictor variables were included in models in continuous form, however, when a continuous variable was not significant, it was transformed into a categorical variable and included in the model if it was significant. Potential variables for possible inclusion in the multivariable models were chosen first by conducting simple associations with the dependent variable, at a p-value cut-off of 0.15. Variables in the final models required a p-value of $p < 0.05$ to remain as a model predictor. Final models were constructed using manual step-wise inclusion to maximize adjusted-R^2 values (or pseudo-R^2), while preserving parsimony. Significant correlations between variables that were at least marginally significant in univariable analyses were investigated. When there were significant correlations between two variables, the variable that was most biologically plausible and produced the largest adjusted R^2 value in multivariable regressions was used.

For each regression analysis, possible confounding effects and interaction between variables was considered. Fit of models was determined by examining

standardized residuals for homogeneity and normality. No models contained significant residual outliers.

All statistical analyses were performed using Stata 11 (StataCorp LP, College Station, Texas).

3.4 Results

3.4.1 Descriptive Statistics of Urinary Arsenic Concentrations

Descriptive statistics of total and speciated As metabolites are presented in Table 3.1, by location and gender, and in Table 3.2 by exposure group. Significant differences between group medians (between locations and between genders within locations, and between genders between locations), determined using permutation analyses, are identified in bold, or with different letter superscript designations.

Table 3.1 Descriptive statistics of urinary arsenic concentrations, total and speciated, by location and gender

	Hubbards		Fall River	
Variable	**Male (n=45)**	**Female (n=62)**	**Male (n=35)**	**Female (n=37)**
Total As (µg/L)				
mean (SD)	70.3 (96.4)	40.6 (76.5)	69.0 (88.5)	53.1 (110.9)
median (range)	27.2 (2.8 - 417.5)	17.8 (0.4 - 477.5)	35.1 (2.8 - 441.9)	20.9 (0.355[1] - 661.1)
TMA (µg/L)				
mean (SD)	42.7 (53.9)	28.3 (16.2)	38.6 (39.6)	32.5 (30.4)
median (range)	31.3 (13.8 - 332.7)a	21.9 (8.7 - 81.5)b	29.6 (13.8 - 236.2)	24.8 (12.1 - 181.9)
As(III) (µg/L)				
mean (SD)	1.8 (3.2)	0.9 (1.4)	0.7 (1.1)	0.7 (0.8)
median (range)	1.0 (0.355[1] - 17.1)a‡	0.5 (0.355[1] - 8.0)b	0.4 (0.355[1] - 4.9)ψ	0.4 (0.355[1] - 2.8)
As(V) (µg/L)				
mean (SD)	2.2 (4.7)	1.2 (1.9)	0.355[1] (1.0)	0.355[1] (1.5)
median (range)	0.355[1] (0.355[1] - 24.4)	0.355[1] (0.355[1] - 8.1)	0.355[1] (0.355[1] - 3.3)	0.355[1] (0.355[1] - 6.0)
MMA(V) (µg/L)				
mean (SD)	2.6 (7.8)	1.3 (1.4)	1.1 (1.1)	1.0 (0.9)
median (range)	1.1 (0.355[1] - 49.9)‡	0.9 (0.355[1] - 6.8)	1.0 (0.355[1] - 4.9)ψ	0.7 (0.355[1] - 4.1)
DMA(V) (µg/L)				
mean (SD)	19.0 (37.6)	12.5 (17.2)	11.0 (12.0)	12.1 (17.0)
median (range)	10.7 (2.6 - 239.4)a‡	6.6 (0.9 - 106.9)b	7.2 (0.355[1] - 64.0)ψ	7.4 (2.4 - 93.5)
AsB (µg/L)				
mean (SD)	42.1 (72.2)	29.7 (72.1)	50.1 (72.5)	37.3 (87.6)
median (range)	14.8 (0.355[1] - 388.0)a	8.2 (0.355[1] - 411.8)b	21.0 (0.355[1] - 340.8)	12.8 (0.355[1] - 489.2)

[1]Data represent the value of one half of the ICP-MS minimum detection limit for total urinary arsenic (0.709 µg/L)

[ab]Different letter designations indicate significant differences ($p < 0.05$) in medians between genders, within locations

‡ψDifferent symbol designations (‡ and ψ) indicate significant differences ($p < 0.05$) in medians within genders, between locations AsB: arsenobetaine, As(III): arsenite, As(V): arsenate, MMA(V): monomethylarsonic acid, DMA(V): dimethylarsinic acid, TMA: total arsenic minus arsenobetaine

SD: standard deviation

Table 3.2 Descriptive statistics of urinary arsenic concentrations, total and speciated, by exposure group based on a 10 μg/L cut-off concentration

Variable	Unexposed (n=105)	Exposed (n=74)
Total (μg/L)		
mean (SD)	44.8 (73.8)	71.6 (110.7)
median (range)	18.7 (0.355[1] - 477.5) ǂ	34.5 (2.6 - 661.1) ǂ
Total - AsB (μg/L)		
mean (SD)	26.9 (17.8)	45.7 (50.4)
median (range)	20.5 (8.7 - 133.5) ǂ	33.2 (13.6 - 332.7) ǂ
As(III) (μg/L)		
mean (SD)	0.355[1] (0.8)	1.7 (2.7)
median (range)	0.2 (0.355[1] - 4.8) ǂ	1.1 (0 - 17.1) ǂ
As(V) (μg/L)		
mean (SD)	0.355[1] (1.1)	2.1 (3.9)
median (range)	0.355[1] (0.355[1] - 6.1) ǂ	0 (0 - 24.2) ǂ
MMA(V) (μg/L)		
mean (SD)	0.8 (0.6)	2.5 (6.0)
median (range)	0.7 (0.355[1] - 3.1) ǂ	1.5 (0.355* - 49.9) ǂ
DMA(V) (μg/L)		
mean (SD)	9.1 (12.7)	19.7 (31.4)
median (range)	6.1 (0.355[1] - 106.9) ǂ	12.1 (2.0 - 239.4) ǂ
AsB (μg/L)		
mean (SD)	35.9 (66.5)	42.3 (85.5)
median (range)	12.8 (0.355[1] - 411.8)	9.9 (0.355[1] - 489.2)

[1]Data represent the value of one half of the ICP-MS minimum detection limit for total urinary arsenic (0.709 μg/L)

ǂDenotes significant differences in medians between exposure categories ($p < 0.05$)AsB: arsenobetaine, As(III): arsenite, As(V): arsenate, MMA(V): monomethylarsonic acid, DMA(V): dimethylarsinic acid, TMA: total arsenic minus arsenobetaine

SD: standard deviation

There were no significant differences in urinary As metabolites between locations. Total urinary As minus AsB (TMA), urinary arsenite, and urinary AsB concentrations were significantly different between males and females in Hubbards, but not in Fall River (Table 3.1). Males from Hubbards were found to have significantly higher concentrations of urinary As(III), MMA(V) and DMA(V) ($p = 0.009$, $p = 0.040$, $p = 0.030$, respectively), than males from Fall River. No significant differences were found between females from different locations. It was found that males from Fall River had significantly higher concentrations of total As and AsB in urine than females from Hubbards ($p = 0.032$ and $p = 0.012$, respectively). Additionally, males from Hubbards had significantly higher concentrations of As(III), MMA(V), and TMA than females from Fall River ($p = 0.057$, $p = 0.056$ and $p = 0.043$, respectively).

For all urinary As categories, except for AsB, the exposed group was found to have a significantly higher concentration in their urine samples than the unexposed group, as expected.

Urine As metabolite concentrations, adjusted by specific gravity values, are presented in Table 3.3, by location and gender, and in Table 3.4, by exposure group.

Table 3.3 Descriptive statistics of specific gravity-adjusted urinary arsenic metabolites, by gender and location

	Hubbards		Fall River	
Variable	**Male (n=42)**	**Female (n=61)**	**Male (n=34)**	**Female (n=35)**
Total As (µg/L)				
mean (SD)	56.09 (69.11)	44.01 (63.87)	84.93 (163.03)	47.66 (82.91)
median (range)	29.92 (2.76 - 328.72)a	22.49 (0.355[1] - 274.75)b	31.64 (4.55 - 884.20)	25.91 (1.53 - 496.14)
TMA (µg/L)				
mean (SD)	28.59 (47.29)	23.50 (28.27)	25.22 (31.54)	20.23 (22.63)
median (range)	18.22 (3.48 - 293.14)	15.09 (0.355[1] - 176.42)	15.43 (3.46 - 171.33)	15.71 (1.73 - 129.02)
As(III) (µg/L)				
mean (SD)	1.62 (2.77)	1.26 (2.98)	0.355[1] (0.92)	0.355[1] (0.69)
median (range)	0.88 (0.355[1] - 15.53)a‡	0.355[1] (0.355[1] - 19.71)b	0.355[1] (0.355[1] - 3.86)ψ	0.355[1] (0.355[1] - 2.51)
As(V) (µg/L)				
mean (SD)	1.81 (3.53)	1.81 (3.60)	0.355[1] (0.91)	0.355[1] (1.50)
median (range)	0.355[1] (0.355[1] - 16.85)	00.355[1] (0.355[1] - 19.96)	0.355[1] (0.355[1] - 3.61)	0.355[1] (0.355[1] - 7.38)
MMA (µg/L)				
mean (SD)	2.61 (7.26)	1.83 (2.80)	0.97 (0.90)	0.95 (0.83)
median (range)	1.00 (0.355[1] - 45.30)	1.00 (0.355[1] - 16.84)	0.76 (0.355[1] - 4.02)	0.81 (0.355[1] - 3.81)
DMA (µg/L)				
mean (SD)	16.59 (33.99)	14.50 (19.90)	9.68 (9.73)	10.94 (12.38)
median (range)	8.64 (2.09 - 217.48)	7.44 (1.15 - 116.69)	6.99 (0.355[1] - 52.61)	7.46 (2.75 - 70.20)
AsB (µg/L)				
mean (SD)	30.83 (46.07)	28.98 (57.37)	64.62 (136.30)	32.92 (65.77)
median (range)	13.79 (0.355[1] - 239.18)a	7.77 (0.355[1] - 306.07)b‡	20.12 (0.355[1]- 712.88)	15.00 (0.355[1] - 367.12)ψ

[1]Data represent the value of one half of the ICP-MS minimum detection limit for urinary arsenic (0.709 µg/L)

[ab]Different letter designations indicate significant differences ($p < 0.05$) in medians between genders, within locations (comparing males and females from same location)

‡ψDifferent symbol designations (‡ and ψ) indicate significant differences ($p < 0.05$) in medians within genders, between locations

AsB: arsenobetaine, As(III): arsenite, As(V): arsenate, MMA(V): monomethylarsonic acid, DMA(V): dimethylarsinic acid, TMA: total arsenic minus arsenobetaine, SD: standard deviation

Table 3.4 Descriptive statistics of specific gravity-adjusted urinary arsenic concentrations, total and speciated, by exposure group based on a 10 μg/L cut-off concentration

Variable	Total population (n=172)	Unexposed (n=100)	Exposed (n=72)
Total (μg/L)			
mean (SD)	55.8 (96.5)	47.4 (97.0)	67.5 (95.1)
median (range)	26.0 (0.355[1] - 884.2)	22.5 (1.2 - 884.2) ǂ	37.1 (0.355[1] - 496.1) ǂ
Total - AsB (μg/L)			
mean (SD)	24.5 (33.7)	17.2 (20.9)	34.0 (43.6)
median (range)	15.9 (0.355[1] - 293.1)	12.0 (0.355[1] - 171.3) ǂ	20.1 (4.2 - 293.1) ǂ
As(III) (μg/L)			
mean (SD)	1.1 (1.3)	0.355[1] (0.7)	1.9 (3.2)
median (range)	<0.355[1] (0.355[1] - 19.7)	0.355[1] (0.355[1] - 3.1) ǂ	1.2 (0.355[1] - 19.7) ǂ
As(V) (μg/L)			
mean (SD)	1.3 (2.9)	0.355[1] (1.6)	2.2 (3.8)
median (range)	<0.355[1] (0.355[1] - 20.0)	0.355[1] (0.355[1] - 11.61) ǂ	0.7 (0.355[1] - 20.0) ǂ
MMA(V) (μg/L)			
mean (SD)	1.7 (4.1)	0.8 (0.6)	2.8 (5.9)
median (range)	0.9 (0.355[1] - 45.3)	0.7 (0.355[1] - 2.9) ǂ	1.4 (0.355[1] - 45.3) ǂ
DMA(V) (μg/L)			
mean (SD)	13.3 (21.8)	8.1 (7.7)	19.9 (30.6)
median (range)	7.5 (0.355[1] - 217.5)	5.8 (0.355[1] - 61.5) ǂ	10.2 (2.8 - 217.5) ǂ
AsB (μg/L)			
mean (SD)	37.8 (80.6)	37.3 (74.4)	38.5 (74.4)
median (range)	12.4 (0.355[1] - 712.9)	13.4 (0.355[1] - 712.9)	10.7 (0.355[1] - 367.1)

[1]Data with an asterisk are the value of one half of the ICP-MS minimum detection limit for urinary arsenic (0.709 μg/L)

ǂSignificant differences in medians between exposure groups ($p < 0.05$)

AsB: arsenobetaine, As(III): arsenite, As(V): arsenate, MMA(V): monomethylarsonic acid, DMA(V): dimethylarsinic acid, TMA: total arsenic minus arsenobetaine, SD: standard deviation

When considering SG-adjusted urinary [As], it was found that males in Hubbards had significantly higher concentrations of total As, arsenite and arsenobetaine, than females. As well, males from Hubbards had significantly higher concentrations of urinary arsenite than males from Fall River. Females in Fall River had significantly higher concentrations of arsenobetaine in urine than females from Hubbards. There were no significant differences in urine As metabolites found between genders in Fall River or between Hubbards and Fall River. It was found that males from Fall River had significantly higher concentrations of total SG-adjusted total As and arsenobetaine in urine than females from Hubbards ($p = 0.053$ and $p = 0.017$, respectively). Individuals in the exposed category had significantly higher urinary [As] for all As metabolites except for AsB.

Due to the complex nature of As metabolism, it is important to understand the relative amounts of each As metabolite found in the urine. For this reason, detailed descriptions of urine As metabolite concentrations in relation to total urinary As, as well as in relation to total As minus arsenobetaine (TMA), are presented in Table 3.5, by location and gender, and in Table 3.6, by exposure. For these tables, all As metabolite concentrations for each participant were first converted to a percent of either the total urinary As, or a percent of TMA. To assist the reader on the interpretation of the numbers in these tables, we provide the following example. In the bottom right corner of Table 3.5, for females in Fall River, 51.1% was the median percentage of total As that was AsB, and the standard deviation for this percentage was 23.1. Significant differences between group

medians, determined using permutation analyses, are again shown in bold, or with different letter superscript designations.

It is shown in Tables 3.5 and 3.6 that the majority of As found in urine was in the form of arsenobetaine, which accounted for between 40 – 60% of total As across both communities. Following this, DMA(V) was the second most abundant As metabolite in urine, accounting for between 23 – 40% of total urinary As and 56 – 63% of TMA. In contrast, As(V), on average, accounted for less than 1% of total urinary As and TMA.

Table 3.5 Descriptive statistics of percentages of each specific gravity-adjusted urinary arsenic metabolite relative to "total urinary arsenic" and "total minus AsB urinary arsenic", by location and gender

	As(III) median % (SD)		As(V) median % (SD)		MMA(V) median % (SD)		DMA(V) median % (SD)		AsB median % (SD)
	%Total	% TMA	%Total	% TMA	%Total	% TMA	%Total	% TMA	%Total
Total	2.7 (3.6)	4.8 (5.1)	<0.1 (4.1)	<0.1 (11.5)	3.3 (4.4)	7.1 (5.6)	27.9 (20.8)	58.5 (26.9)	49.3 (26.8)
Hubbards	3.4 (4.0) ǂ	5.9 (5.3) ǂ	<0.1 (11.1)	<0.1 (13.2)	5.2 (4.8) ǂ	9.1 (5.5) ǂ	37.1 (22.4) ǂ	62.9 (30.1) ǂ	41.7 (28.9ǂ
Male	3.5 (3.4)	6.1 (5.6)	<0.1 (7.5)	<0.1 (10.6)	3.6 (4.4)	8.4 (5.6)	28.0 (19.7)	60.7 (17.2)	45.2 (25.1)
Female	3.1 (4.5)	5.7 (5.0)	<0.1 (13.2)	<0.1 (15.0)	5.8 (5.1)	9.5 (5.6)	39.7 (24.2)	63.8 (37.3	39.7 (31.6)
Fall River	1.9 (2.7) ǂ	4.1 (4.7) ǂ	<0.1 (5.5)	<0.1 (7.8)	2.7 (3.5) ǂ	6.2 (5.6) ǂ	24.5 (17.1) ǂ	55.8 (21.8) ǂ	52.3 (22.9) ǂ
Male	1.8 (2.8)	4.1 (4.7)	<0.1 (5.7)	<0.1 (8.3)	2.7 (2.9)	5.8 (5.3)	23.3 (15.8)	56.3 (17.9)	59.4 (22.8)
Female	2.0 (2.6)	4.1 (4.8)	<0.1 (5.3)	<0.1 (7.3)	2.9 (3.9)	6.6 (5.9)	24.5 (18.1)	55.7 (24.6)	51.1 (23.1)

ǂ Indicates significant differences ($p < 0.05$) in medians between locations

Table 3.6 Descriptive statistics of percentages of each specific gravity-adjusted urinary arsenic metabolite relative to "total urinary arsenic" and "total minus AsB urinary arsenic", by exposure group

	As(III) median % (SD)		As(V) median % (SD)		MMA(V) median % (SD)		DMA(V) median % (SD)		AsB median % (SD)
	%Total	%TMA	%Total	%TMA	%Total	%TMA	%Total	%TMA	%Total
Total	2.7 (3.6)	4.8 (5.1)	<0.1 (4.1)	<0.1 (11.5)	3.3 (4.4)	7.1 (5.6)	27.9 (20.8)	58.5 (26.9)	49.3 (26.8)
Unexposed	1.8 (3.2) ǂ	2.6 (5.1) ǂ	<0.1 (7.4) ǂ	<0.1 (10.3) ǂ	3.1 (4.4) ǂ	6.6 (5.9)	25.2 (18.9) ǂ	59.5 (32.8)	52.4 (25.0) ǂ
Exposed	4.3 (3.9) ǂ	6.0 (5.0) ǂ	<0.1 (11.1) ǂ	<0.1 (12.7) ǂ	5.4 (4.4) ǂ	7.6 (5.2)	37.4 (22.4) ǂ	56.9 (17.1)	33.5 (26.9) ǂ

ǂ Indicates significant differences between exposure categories for that As metabolite.

Calculations to determine the percent that each As metabolite contributed to total As was performed using specific gravity-adjusted values. However, because all observations were adjusted using the same formula, percentages of total As would be identical when using both unadjusted or adjusted urinary As values.

It was found that percentage of urinary As(III), MMA(V), and DMA(V) concentrations were all significantly higher in Hubbards than in Fall River (% of total and % TMA), and conversely, urinary AsB was significantly higher in Fall River. Additionally, percentage of total urinary As(III), As(V), MMA(V), and DMA(V) concentrations were significantly higher in the exposed group, whereas percentage of urinary AsB was significantly higher in the unexposed group. Percentage of TMA for As(III) and As(V) were also significantly higher in the exposed category than the unexposed category, while MMA(V) and DMA(V) were not different.

Table 3.7 shows all variables that were found to be at least moderately significant ($p < 0.15$) in univariable analyses. Additionally, Table 3.8 shows correlation coefficients between the variables found to be moderately significant ($p < 0.015$) in any of the univariable regression analyses.

Table 3.7 Univariable regression analysis results, by specific gravity-adjusted urine arsenic metabolites, for variables with at least moderate significance (p < 0.15)

		Total As		Total - AsB	
Variable	**Variable Description**	**Coef. (95% CI)**	**p-value**	**Coef. (95% CI)**	**p-value**
Age (>50)	0 - <50, 1 >50	0.514 (0.164 - 0.865)	0.004	0.238 (-0.054 - 0.530)	0.109
Age (years)	Range: 28 – 83 years	0.014 (0.002 - 0.027)	0.028	-	-
Chicken (oz.)	Consumption range: 0 -16 oz.	-0.041 (-0.091 - 0.010)	0.112	-	-
Gender	0 – Male, 1 - Female	-0.302 (-0.643 - 0.039)	0.082	-	-
Rice (cups)	Consumption range: 0 – 7 cup	-	-	-0.195 (0.430 - 0.040)	0.103
Seafood (oz.)	Consumption range: 0 - 24 oz.	0.116 (0.086 - 0.146)	< 0.001	0.081 (0.058 - 0.104)	< 0.001
Seafood (Y/N)	Seafood consumed: 37% yes	1.110 (0.797 - 1.423)	< 0.001	0.707 (0.448 - 0.966)	< 0.001
Softener	Water softener used: 22% yes	-	-	0.335 (0.025 - 0.645)	0.034
Three foods* (μg)	Range: 0 - 2,021 μg	0.001 (0.001 - 0.002)	< 0.001	0.001 (0.001 - 0.001)	< 0.001
Water [As]	Range: <MDL - 309 μg/L	0.008 (0.004 - 0.013)	< 0.001	0.011 (0.008 - 0.014)	< 0.001
Water As high	>10 μg/L: 40%	0.425 (0.083 - 0.767)	0.015	0.590 (0.329 - 0.850)	< 0.001
Water [As(III)]	Range: 0.07 - 249 μg/L	0.017 (0.002 - 0.032)	0.031	0.009 (0.005 - 0.013)	< 0.001
Water [As(V)]	Range: 0.5 - 236 μg/L	0.006 (0.0005 - 0.012)	0.033	-	-

*Three foods: total arsenic consumed from rice, chicken and seafood combined (μg)
Coefficients are in natural log format
AsB: arsenobetaine, As(III): arsenite, As(V): arsenate, 95% CI: 95% confidence interval

Table 3.7 Univariable regression analyses results, by specific gravity-adjusted urine arsenic metabolites, for variable with at least moderate significance (p < 0.05) (continued)

		As(III)		As(V)	
Variable	**Variable Description**	**Coef. (95% CI)**	**p-value**	**Coef. (95% CI)**	**p-value**
Age (>50)	0 - <50, 1 >50	-	-	0.434 (-0.037 - 0.906)	0.07
Age (years)	Range: 28 – 83 years	-	-	0.013 (-0.004 - 0.030)	0.127
Chicken (Y/N)	Chicken consumed: 54% yes	-	-	-0.495 (-0.990 - 0.001)	0.05
Location	0 - Hubbards 1 - Fall River	-0.432 (-0.788 to -0.077)	0.018	-	-
Rice (Y/N)	Rice consumed: 4% yes	-0.469 (-0.995 - 0.058)	0.08	-	-
Seafood (oz.)	Consumption range: 0 - 24 oz.	0.098 (0.053 - 0.142)	< 0.001	0.074 (0.020 - 0.127)	0.008
Seafood (Y/N)	Seafood consumed: 37% yes	0.661 (0.298 - 1.023)	< 0.001	-	-
Softener	Water softener used: 22% yes	0.481 (0.096 - 0.866)	0.015	-	-
Three foods* (μg)	Range: 0 - 2,021 μg	0.001 (0.0005 - 0.002)	< 0.001	0.0009 (0.0002 - 0.0015)	0.011
Water [As]	Range: <MDL - 309 μg/L	0.012 (0.009 - 0.015)	< 0.001	0.008 (0.005 - 0.012)	< 0.001
Water As (>10 μg/L)	>10 μg/L: 40%	0.841 (0.527 - 1.155)	< 0.001	0.592 (0.134 - 1.050)	0.012
Water [As(III)]	Range: 0.07 - 249 μg/L	0.018 (0.006 - 0.029)	0.004	-	-
Water [As(V)]	Range: 0.5 - 236 μg/L	0.010 (0.006 - 0.013)	< 0.001	0.008 (0.004 - 0.012)	< 0.001
Water Consumed (L)	Range: 0 - 7.7 L	0.155 (0.036 - 0.275)	0.012	0.172 (0.005 - 0.339)	0.044
Water pH	Range: 3.2 - 8.4	0.480 (0.261 - 0.699)	< 0.001	-	-

*Three foods: total arsenic consumed from rice, chicken and seafood combined (μg)
Coefficients in natural log format
As(III): arsenite, As(V): arsenate, 95% CI: 95% confidence interval

Table 3.7 Univariable regression analyses results, by specific gravity-adjusted urine arsenic metabolites, for variable with at least moderate significance (p < 0.05) (continued)

		DMA(V)		MMA(V)	
Variable	**Variable Description**	**Coef. (95% CI)**	**p-value**	**Coef. (95% CI)**	**p-value**
Age (>50)	0 - <50, 1 >50	-	-	0.241 (-0.056 - 0.538)	0.11
Location	0 - Hubbards 1 - Fall River	-	-	-0.301 (-0.588 - -0.014)	0.04
Rice (cups)	Consumption range:0 - 7	-0.214 (-0.444 - 0.016)	0.068	-	-
Rice (Y/N)	Rice consumed: 4% yes	-0.351 (-0.743 - 0.041)	0.079	-	-
Seafood (oz.)	Consumption range: 0 - 24 oz.	0.077 (0.055 - 0.100)	< 0.001	0.037 (0.008 - 0.067)	0.014
Seafood (Y/N)	Seafood consumed: 37% yes	-	-	0.286 (-0.011 - 0.054)	0.059
Softener	Water softener used: 22% yes	0.262 (-0.033 - 0.557)	0.082	-	-
Three foods* (μg)	Range: 0 - 2,021 μg	0.0009 (0.0001 - 0.0012)	< 0.001	0.0004 (0.0001 - 0.0008)	0.019
Water [As]	Range: <MDL - 309 μg/L	0.012 (0.009 - 0.015)	< 0.001	-	-
Water As (>10 μg/L)	>10 μg/L: 40%	0.693 (0.438 - 0.947)	< 0.001	0.706 (0.447 - 0.965)	< 0.001
Water [As(III)]	Range: 0.07 - 249 μg/L	0.015 (0.002 - 0.029)	0.021	0.020 (0.007 - 0.033)	0.004
Water [As(V)]	Range: 0.5 - 236 μg/L	0.010 (0.007 - 0.014)	< 0.001	0.010 (0.006 - 0.013)	< 0.001
Water Consumed (L)	Range: 0 - 7.7 L	-	-	0.118 (0.018 - 0.218)	0.021
Water pH	Range: 3.2 - 8.4	0.397 (0.225 - 0.570)	< 0.001	0.481 (0.301 - 0.661)	< 0.001

*Three foods: total arsenic consumed from rice, chicken and seafood combined (μg)
Coefficients in natural log format
DMA(V): dimethylarsinic acid, MMA(V): monomethylarsonic acid, As(III): arsenite, As(V): arsenate, 95% CI: 95% confidence interval

Table 3.7 Univariable regression analyses results, by specific gravity-adjusted urine arsenic metabolites, for variable with at least moderate significance (p < 0.05) (continued)

		AsB	
Variable	**Variable Description**	**Coef. (95% CI)**	**p-value**
Age (>50)	0 - <50, 1 >50	0.653 (0.132 - 1.174)	0.014
Chicken (oz.)	Consumption range: 0 - 16 oz.	-0.099 (-0.172 - -0.023)	0.008
Chicken (Y/N)	Chicken consumed: 54% yes	-0.406 (-0.894 - 0.082)	0.102
Gender	0 – Male 1 - Female	-0.452 (-0.938 - 0.035)	0.069
Location	0 - Hubbards 1 - Fall River	0.468 (-0.023 - 0.960)	0.062
Seafood (oz.)	Consumption range: 0 - 24 oz.	0.126 (0.086 - 0.168)	< 0.001
Seafood (Y/N)	Seafood consumed: 37% yes	1.422 (0.980 - 0.1863)	< 0.001
Three foods[1] (μg)	Range: 0 - 2,021 μg	0.002 (0.001 - 0.002)	< 0.001
Water [As]	Range: <MDL - 309 μg/L	0.0004 (-0.006 – 0.007)	0.888[2]
Water (>10 μg/L)	>10 μg/L: 40%	-0.088 (-0.583 – 0.408)	0.727[2]
Water [As(III)]	Range: 0.07 - 249 μg/L	0.014 (-0.009 – 0.037)	0.228[2]
Water [As(V)]	Range: 0.5 - 236 μg/L	-0.0005 (-0.009 – 0.008)	0.910[2]

[1]Three food: total arsenic consumed from rice, chicken and seafood combined (μg)
[2]p-values above 0.15 for AsB variables added to highlight the non-association between urinary AsB and water As characteristics
Coefficients in natural log format
AsB: arsenobetaine, As(III): arsenite, As(V): arsenate, 95% CI: 95% confidence interval

Table 3.8 Correlation coefficients between variables found to be at least moderately significant (p < 0.15) in univariable regression analysis of urinary arsenic metabolites

Variable	Age >50 years (Y/N)	Age (years)	Estimated Chicken consumed (oz.)	Chicken consumed (Y/N)	Gender (M/F)
Age >50 years (Y/N)	1.0000*				
Age (years)	0.7541*	1.0000*			
Estimated chicken consumed (oz.)	-0.1301	-0.175*	1.0000*		
Chicken consumed (Y/N)	-0.0028	-0.091	0.7978*	1.0000*	
Gender (M/F)	-0.0187	-0.0803	-0.0623	0.0079	1.0000*
Location (H/FR)	-0.0798	-0.104	0.0159	-0.0461	-0.0647
Water pH	-0.2763*	-0.3522*	0.0538	0.0627	0.0338
Estimated rice consumed (cups)	-0.134	-0.1058	0.0045	-0.0147	-0.0951
Rice consumed (Y/N)	-0.1475	-0.164	0.0414	-0.0053	-0.015
Estimated seafood consumed (oz.)	0.0971	0.1319	-0.2309*	-0.2452*	-0.0812
Seafood consumed (Y/N)	0.1246	0.1252	-0.1597*	-0.1108	-0.0816
Water softener used (Y/N)	-0.0093	-0.0644	0.1153	0.0232	-0.0711
Estimated As consumed from chicken, rice & seafood (μg)	**0.0824**	**0.1195**	**-0.2205***	**-0.2385***	**-0.0909**
Water [As] (μg/L)	**0.0767**	**0.0643**	**-0.0229**	**-0.0055**	**-0.0356**
Water [As] >10 μg /L (Y/N)	**-0.063**	**-0.1367**	**0.1286**	**0.0911**	**-0.0188**
Water [As(III)) (μg /L)	**0.2638**	**0.1807**	**-0.146**	**-0.1791**	**-0.0847**
Water [As(V)] (μg /L)	**0.1759**	**0.1876**	**-0.0578**	**-0.0041**	**0.0145**
Estimated As consumed from water (μg)	**0.1174**	**0.0379**	**-0.0028**	**0.0874**	**0.0629**

*Data with an asterisk were significantly correlated at the p <0.05 level
As(III): arsenite, As(V): arsenate

Table 3.8 Correlation coefficients between variables found to be at least moderately significant (p < 0.15) in univariable regression analysis of urinary arsenic metabolites (continued)

Variable	Location (H/FR)	Water pH	Estimated rice consumed (cups)	Rice consumed (Y/N)	Estimated seafood consumed (oz.)
Location (H/FR)	1.0000*				
Water pH	0.0317	1.0000*			
Estimated rice consumed (cups)	0.0544	-0.1221	1.0000*		
Rice consumed (Y/N)	0.1172	-0.0192	0.7851*	1.0000*	
Estimated seafood consumed (oz.)	0.0826	-0.0548	0.0033	0.0825	1.0000*
Seafood consumed (Y/N)	0.1524*	-0.0182	0.0345	0.1172	0.731*
Water softener used (Y/N)	0.2535*	0.2155*	-0.0276	-0.0063	0.1313
Estimated As consumed from chicken, rice & seafood (μg)	0.0878	-0.0665	0.1015	0.1597*	0.9951*
Water [As] (μg/L)	-0.1169	0.2064*	-0.1158	-0.1215	0.2619*
Water [As] >10 μg /L (Y/N)	0.0474	0.3725*	-0.1518*	-0.1023	0.1033
Water [As(III)) (μg /L)	-0.2731	-0.1083	-0.1124	-0.1174	0.6563*
Water [As(V)] (μg /L)	-0.0955	0.1129	-0.0779	-0.0767	0.3112*
Estimated As consumed from water (μg)	-0.1319	0.2303*	-0.0684	-0.0724	0.1909*

*Data with an asterisk were significantly correlated at the p <0.05 level
As(III): arsenite, As(V): arsenate

Table 3.8 Correlation coefficients between variables found to be at least moderately significant (p < 0.15) in univariable regression analysis of urinary arsenic metabolites (continued)

Variable	Seafood consumed (Y/N)	Water softener used (Y/N)	Estimated As consumed from chicken, rice & seafood (μg)	Water [As] (μg/L)
Seafood consumed (Y/N)	1.0000*			
Water softener used (Y/N)	0.139	1.0000*		
Estimated As consumed from chicken, rice & seafood (μg)	0.7306*	0.129	1.0000*	
Water [As] (μg/L)	0.1961*	-0.0362	0.2496*	1.0000*
Water [As] >10 μg /L (Y/N)	0.0815	0.0439	0.0893	0.4958*
Water [As(III)) (μg /L)	0.3581	-0.1396	0.6335*	0.9538*
Water [As(V)] (μg /L)	0.1961	-0.1699	0.3057*	0.7035*
Estimated As consumed from water (μg)	0.1044	-0.0274	0.1836*	0.6665*

*Data with an asterisk were significantly correlated at the p <0.05 level
As(III): arsenite, As(V): arsenate

Table 3.8 Correlation coefficients between variables found to be at least moderately significant (p < 0.15) in univariable regression analysis of urinary arsenic metabolites (continued)

Variable	Water [As] >10 μg /L (Y/N)	Water [As(III)) (μg /L)	Water [As(V)] (μg /L)	Estimated As consumed from water (μg)
Water [As] >10 μg /L (Y/N)	1.0000*			
Water [As(III)) (μg /L)	0.2303	1.0000*		
Water [As(V)] (μg /L)	0.2957*	0.1608	1.0000	
Estimated As consumed from water (μg)	0.2969*	0.6803*	0.8058*	1.0000*

*Data with an asterisk were significantly correlated at the p <0.05 level
As(III): arsenite, As(V): arsenate

3.4.2 Analytical Statistical Analyses of Factors Associated with Urinary Arsenic Concentration

In the linear regression analyses that were run to determine demographic and lifestyle factors associated with total urinary As, TMA, As(III), As(V), MMA(V), DMA(V), and AsB, many predictors were similar across the different regression analyses. For ease of comparison, all regression statistics are presented together in Table 3.9. All regression coefficients and confidence intervals are presented in natural log format. All regression models met assumptions of independence, linearity and homoscedasticity. In addition, a second linear regression analysis with total urinary As (and TMA for comparison purposes) as the outcome (Table 3.10) was performed to examine specifically the relationship between total urinary As and water [As], after controlling for household income and As consumed from food. This relationship was of particular interest to provide data regarding the decision by the Nova Scotia Department of Environment to lower the [As] in drinking water standard to 10 μg/L.

Table 3.9 Multivariable linear regression analyses for total and speciated, natural log-transformed, specific gravity-adjusted urinary arsenic concentration

Metabolite	Total As			TMA		
Variable	**Coef. (Robust St. Error)**	**95% CI**	**p-value**	**Coef. (Robust St. Error)**	**95% CI**	**p-value**
Participant age (>50 years)	0.50 (0.16)	0.18 - 0.83	0.003	-	-	-
Seafood consumed (oz.)	0.11 (0.01)	0.09 - 0.14	<0.001	0.09 (0.01)	0.06 - 0.11	<0.001
Water [As] Category			0.004*			0.033*
1 - 0 - 25th percentile	Referent			Referent		
2 - 25 - 50th percentile	-0.17 (0.23)	-0.62 - 0.28	0.460	-0.22 (0.17)	-0.56 - 0.12	0.195
3 - 50 - 75th percentile	0.43 (0.21)	0.02 - 0.84	0.041	0.13 (0.18)	-0.24 - 0.49	0.487
4 - 75 - 100th percentile	0.51 (0.23)	0.09 - 0.14	<0.001	0.42 (0.21)	0.003 - 0.83	0.049
Water pH	-	-	-	0.32 (0.12)	0.09 - 0.55	0.008
Constant	1.98 (0.32)	1.35 - 2.61	<0.001	0.51 (0.66)	-0.79 - 1.81	0.438
n	171			141		
Adjusted-R^2	0.3583			0.4397		

*Overall p-value for categorical variable
Coefficients in natural log format; Negative coefficients become positive when back-transformed
TMA: total arsenic minus arsenobetaine, 95% CI: 95% confidence interval

- Total urinary arsenic model with back-transformed coefficients:
  Total urinary [As] = 7.2 + [1.7*(participant age > 50 years)] + [1.1*(seafood consumed in oz.)] + [0.8*(water [As] categ. 2)] + [1.5*(water [As] categ. 3)] + [1.7*(water [As] categ. 4)] + error

- TMA model with back-transformed coefficients:
  [TMA] = 1.7 + [1.1*(seafood consumed in oz.)] + [0.8*(water [As] categ. 2)] + [1.1* (water [As] categ. 3)] +[1.5*(water [As] categ. 4)] + [1.4*(water pH)] + error

Table 3.9 Multivariable linear regression analyses for total and speciated, natural log-transformed, specific gravity-adjusted urinary arsenic concentration (continued)

Metabolite	As(III)			As(V)		
Variable	**Coef. (Robust St. Error)**	**95% CI**	**p-value**	**Coef. (Robust St. Error)**	**95% CI**	**p-value**
Chicken consumed (y/n)	-	-	-	-0.75 (0.35)	-1.16 to -0.34	0.001
Seafood consumed (oz.)	0.09 (0.03)	0.02 - 0.15	0.010	-	-	-
Water [As] Category			<0.001*			<0.001*
1 - 0 - 25th percentile	Referent			Referent		
2 - 25 - 50th percentile	0.03 (0.19)	-0.35 - 0.41	0.866	-0.33 (0.35)	-1.03 - 0.38	0.355
3 - 50 - 75th percentile	0.61 (0.18)	0.25 - 0.98	0.001	0.49 (0.33)	-0.18 - 1.16	0.150
4 - 75 - 100th percentile	1.17 (0.22)	0.73 - 1.62	<0.001	0.93 (0.38)	0.17 - 1.69	0.018
Constant	-0.63 (0.15)	-0.92 to -0.34	<0.001	0.96 (0.33)	0.29 - 1.63	0.006
n	93			53		
Adjusted-R^2	0.4551			0.3592		

*Overall p-value for categorical variable
Coefficients in natural log format; Negative coefficients become positive when back-transformed
As(III): arsenite, As(V): arsenate, 95% CI: 95% confidence interval

Urinary [As(III)] model with back-transformed coefficients:

Urinary [As(III)] = 0.5 + [1.1*(seafood consumed (oz.))] + [1.0*(water [As] categ. 2)] + [1.8*(water [As] categ. 3)] + [3.2*(water [As] categ. 4)] + error

- Urinary [As(V)] model with back-transformed coefficients:

Urinary [As(V)] = 2.6 + [0.5*(chicken consumed)] + [0.7*(water [As] categ. 2)] + [1.6*(water [As] categ. 3)] + [2.5*(water [As] categ. 4)] + error

Table 3.9 Multivariable linear regression analyses for total and speciated, natural log-transformed, specific gravity-adjusted urinary arsenic concentration (continued)

Metabolite	MMA(V)			DMA(V)		
Variable	**Coef. (Robust St. Error)**	**95% CI**	**p-value**	**Coef. (Robust St. Error)**	**95% CI**	**p-value**
Location	0.35 (0.19)	-0.03 - 0.73	0.074	0.17 (0.20)	-0.23 - 0.57	0.403
Seafood consumed (oz.)	-	-	-	0.09 (0.01)	0.06 - 0.11	<0.001
Water [As] Category			<0.001*			0.002*
1 - 0 - 25th percentile	Referent			Referent		
2 - 25 - 50th percentile	0.21 (0.14)	-0.07 - 0.49	0.139	0.05 (0.19)	-0.33 - 0.43	0.787
3 - 50 - 75th percentile	0.74 (0.18)	0.38 - 1.09	<0.001	0.43 (0.25)	-0.07 - 0.93	0.092
4 - 75 - 100th percentile	1.34 (0.38)	0.59 - 2.09	0.001	1.01 (0.29)	0.43 - 1.58	0.001
Location*Water [As] Category			0.018*			0.022*
2	-0.35 (0.28)	-0.90 - 0.21	0.215	-0.12 (0.31)	-0.73 - 0.50	0.710
3	-0.70 (0.29)	-1.27 to -0.12	0.019	-0.24 (0.30)	-0.83 - 0.36	0.433
4	-1.23 (0.45)	-2.12 to -0.34	0.007	-0.97 (0.32)	-1.61 to -0.33	0.003
Water pH	0.26 (0.12)	0.03 - 0.49	0.030	0.24 (0.10)	0.04 - 0.44	0.022
Constant	-1.96 (0.68)	-3.31 to -0.62	0.005	0.11 (0.61)	-1.10 - 1.32	0.854
n	119			141		
Adjusted-R^2	0.4044			0.5517		

*Overall p-value for categorical variable
Coefficients in natural log format; Negative coefficients become positive when back-transformed
Location*Water [As] Category: interaction term between location and water arsenic concentration category
MMA(V): monomethylarsonic acid, DMA (V): dimethylarsinic acid, 95% CI: 95% confidence interval, Location: Hubbards = 0, Fall River = 1

Table 3.9 Multivariable linear regression analyses for total and speciated, natural log-transformed, specific gravity-adjusted urinary arsenic concentration (continued)

- Urinary [MMA(V)] model with back-transformed coefficients:

  Urinary [MMA(V)] = 0.1 + [1.4*(location)] + [1.2*(water [As] categ. 2)] + [2.1*(water [As] categ. 3)] + [3.8*(water [As] categ. 4)] + [0.7*(location*water [As] categ. 2)] + [0.5*(location*water [As] categ. 3)] + [0.3*(location*water [As] categ. 4)] + [1.3*(pH)] + error

- Urinary [DMA(V)] model with back-transformed coefficients:

  Urinary [DMA(V)] = 1.1 + [1.2*(location)] + [1.1*(seafood consumed (oz.))] + [1.1*(water [As] categ. 2)] + [1.5*(water [As] categ. 3)] + [2.8*(water [As] categ. 4)] + [0.9*(location*water [As] categ. 2)] + [0.8*(location*water [As] categ. 3)] + [0.4*(location*water [As] categ. 4)] + [1.3*(pH)] + error

Table 3.9 Multivariable linear regression analyses for total and speciated, natural log-transformed, specific gravity-adjusted urinary arsenic concentration (continued)

Metabolite	**AsB**		
Variable	**Coef. (Robust St. Error)**	**95% CI**	**p-value**
Participant age (years)	0.02 (0.01)	0.01 - 0.04	0.009
Seafood consumed (y/n)	1.39 (0.24)	0.91 - 1.85	<0.001
Constant	0.81 (0.54)	-0.25 - 1.88	0.133
n	142		
Adjusted-R^2	0.2669		

Coefficients in natural log format; Negative coefficients become positive when back-transformed
AsB: arsenobetaine, 95% CI: 95% confidence interval

- Urinary AsB model with back-transformed coefficients:

Urinary [AsB] = 2.3 + [1.0*(participant age (>50 years))] + [4.0*(seafood consumed (y/n)] + error

Table 3.10 Multivariable linear regression analysis for total, and total minus arsenobetaine (TMA), natural log-transformed, specific gravity-adjusted urinary arsenic concentration to evaluate the Nova Scotia Department of Environment water standard of 10 μg/L of arsenic in water

Metabolite	Total As			TMA		
Variable	**Coef. (Robust St. Error)**	**95% CI**	**p-value**	**Coef. (Robust St. Error)**	**95% CI**	**p-value**
Income Category			0.996*			0.376*
1 - < $30,000/year	Referent			Referent		
2 - $30,000 - $50,000/year	-0.02 (0.32)	-0.64 - 0.60	0.952	-0.28 (0.25)	-0.77 - 0.29	0.259
3 - > $50,000/year	-0.02 (0.28)	-0.57 - 0.53	0.930	-0.22 (0.16)	-0.54 - 0.10	0.176
Food As Intake (μg)	0.0006 (0.0001)	0.0004 - 0.0008	<0.001	0.0003 (0.0001)	0.0001 - 0.0005	0.002
Water [As] Category			0.047*			0.002*
1 - <10 μg/L	Referent			Referent		
2 - 10 - 20 μg/L	0.14 (0.24)	-0.34 - 0.62	0.572	0.15 (0.15)	-0.15 - 0.45	0.333
3 - 20 - 50 μg/L	0.30 (0.24)	-0.18 - 0.77	0.214	0.60 (0.21)	0.17 - 1.02	0.006
4 - > 50 μg/L	0.77 (0.28)	0.21 - 1.32	0.007	1.06 (0.36)	0.34 - 1.78	0.004
Constant	2.95 (0.26)	2.45 - 3.46	<0.001	2.62 (0.16)	2.31 - 2.91	<0.001
n	156			137		
Adjusted-R2	0.1826			0.3021		

*Overall p-value for categorical variable
Coefficients in natural log format; Negative coefficients become positive when back-transformed
TMA: Total arsenic minus arsenobetaine, 95% CI: 95% confidence interval

- Total urinary [As] model with back-transformed coefficients:

  Total urinary [As] = 19.1 + [1.0*(income categ. 2)] + [1.0*(income categ. 3)] + [1.0*(food As intake (μg)*)] + [1.2*(water [As] categ. 2*)] + [1.4*(water [As] categ. 3*)] + [2.2*(water [As] categ. 4)] + error

Table 3.10 Multivariable linear regression analysis of natural log-transformed, specific gravity-adjusted total urinary arsenic concentration (μg/L) and total urinary arsenic minus arsenobetaine (TMA) to evaluate the Nova Scotia Department of Environment water standard of 10 μg/L of arsenic in water (continued)

- Total arsenic minus arsenobetaine model with back-transformed coefficients:

  [TMA] = 13.7 + [0.8*(income categ. 2)] + [0.8*(income categ. 3)] + [1.0*(food As intake (μg)*)] + [1.2*(water [As] categ. 2*)] + [1.8*(water [As] categ. 3*)] + [2.9*(water [As] categ. 4)] + error

In the logistic regression analyses for urinary As metabolites with <80% of observations having a concentration >MDL (0.709 μg/L), the significant demographic and lifestyle factors associated with As(III), As(V), and MMA(V) concentrations are presented in Table 3.11.

Table 3.11 Logistic regression analysis for urinary As(III), As(V), and MMA(V) with the ICP-MS MDL (0.709 μg/L) as a cut-off concentration

Metabolite	As(III)			As(V)		
Variable	**Odds Ratio (Robust St. Error)**	**95% CI**	**p-value**	**Odds Ratio (Robust St. Error)**	**95% CI**	**p-value**
Water [As] Category			<0.001*			<0.001*
1 - 0 - 25th percentile	Referent			Referent		
2 - 25 - 50th percentile	2.14 (1.66)	0.47 - 9.81	0.325	2.84 (1.73)	0.87 - 9.35	0.085
3 - 50 - 75th percentile	14.26 (9.51)	3.86 - 52.72	<0.001	5.72 (3.48)	1.74 - 18.84	0.004
4 - 75 - 100th percentile	12.61 (9.40)	2.92 - 54.37	0.001	8.70 (5.02)	2.81 - 26.96	0.001
Seafood consumed (oz.)	1.66 (0.68)	0.66 - 3.68	0.313	-	-	-
Chicken consumed (y/n)	-	-	-	2.01 (0.74)	0.98 - 4.13	0.057
Location	-	-	-	0.42 (0.17)	0.19 - 0.91	0.028
Constant	0.09 (0.05)	0.03 - 0.27	<0.001	0.10 (0.05)	0.03 - 0.29	<0.001
n	172			172		
Pseudo-R^2	0.1838			0.1347		

* Overall p-value for categorical variable
Coefficients in natural log format; Negative coefficients become positive when back-transformed
As(III): arsenite, As(V), arsenate, 95% CI: 95% confidence interval, Location: Hubbards = 0, Fall River =1

- Urinary [As(III)] model using back-transformed coefficients:
ln[p(urinary [As(III)] >MDL)/1 – p(urinary [As(III)] >MDL)] = -2.4 + [0.8*(water [As] categ. 2)] + [2.7*(water [As] categ. 3)] + [2.5*(water [As] categ. 4)] + [0.4*(seafood (oz.)]

- Urinary [As(V)] model using back-transformed coefficients
ln[p(urinary [As(V)] >MDL)/1 – p(urinary [As(V)] >MDL)] = -2.3 + [1.1*(water [As] categ. 2)] + [1.7*(water [As] categ. 3)] + [2.2*(water [As] categ. 4)] + [0.7*(chicken (y/n)] + [-0.9*(location)]

Table 3.11 Logistic regression analysis for urinary As(III), As(V), and MMA(V) with the ICP-MS MDL (0.709 μg/L) as a cut-off concentration (continued)

Metabolite	**MMA(V)**		
Variable	**Odds Ratio (Robust St. Error)**	**95% CI**	**p-value**
Water [As] Category			0.002*
1 - 0 - 25th percentile	Referent		
2 - 25 - 50th percentile	5.29 (3.40)	1.50 - 18.61	0.009
3 - 50 - 75th percentile	13.92 (9.95)	3.43 - 56.50	<0.001
4 - 75 - 100th percentile	10.85 (7.97)	2.57 - 45.77	0.001
Location	4.77 (3.88)	0.97 - 25.53	0.055
Location*Water [As] Category			0.103*
2	0.18 (0.19)	0.02 - 1.35	0.095
3	0.14 (0.02 - 1.15	0.02 - 1.15	0.066
4	0.08 (0.08)	0.01 - 0.62	0.015
Water pH	1.63 (0.02)	0.93 - 2.86	0.091
Constant	162 0.1436		
n			0.103
Pseudo-R^2	0.18 (0.19)	0.02 - 1.35	0.095

Location*Water [As] Category: interaction term between location and water arsenic concentration category
*Overall p-value for categorical variable
Coefficients in natural log format; Negative coefficients become positive when back-transformed
MMA(V): monomethylarsonic acid, 95% CI: 95% confidence interval, Location: Hubbards = 0, Fall River =1

- Urinary [MMA(V)] model using back-transformed coefficients:
  ln[p(urinary [MMA(V)] >MDL)/1 – p(urinary [MMA(V)] >MDL)] = -4.5 + [1.6*(location)] + [1.7*(water [As] categ. 2)] + [2.6*(water [As] categ. 3)] + [2.4*(water [As] categ. 4)] + [-1.7*(water [As] categ. 2*location)] + [-2.0*(water [As] categ. 3*location)] + [-2.5*(water [As] categ. 4*location)] + [0.5*(water pH]

3.4.3 Factors Associated with Total and Total Minus Arsenobetaine (TMA) Urinary Arsenic Concentration (Table 3.9)

Based on the coefficients in Table 3.9, and an increase in seafood consumption from the 25th to the 75th percentile (0 – 4 oz. or 113.2 g), the predicted total urinary As concentration increased by 1.6 times (95% CI: 1.4 – 1.8). As well, participants who were older than 50 years of age were predicted to have a total urinary As concentration of approximately 1.7 times (95% CI: 1.2 – 2.3) higher than younger participants. Total urinary [As] was found to increase by 1.5 times (95% CI: 1.02 – 2.31) and 1.7 times (95% CI: 1.1 – 2.5) for participants with a water [As] in the third and fourth quartiles, respectively, compared to those in the lowest quartile.

Linear regression analysis found that water [As] was a significant predictor of urinary [TMA]. Participants with water [As] in the highest quartile of all samples were predicted to have a urinary [TMA] of 1.5 times (95% CI: 1.1 – 2.2) higher than those in the lowest quartile of water [As] samples. Participants with water samples in category 2 and 3 were not predicted to have a significantly different [TMA] in urine than those in category 1. For an increase in seafood consumption from the 25th to the 75th percentile (0 – 4 oz. or 113.2 g), the predicted concentration of urinary TMA increased by 1.4 times (95% CI: 1.3 – 1.5). Additionally, a predicted increase of 1.4 times (95% CI: 1.2 – 1.7) the urinary [TMA] was found for participants with a water pH at the 75th percentile (pH = 6.6) compared to those with a pH at the 25th percentile (pH = 5.5).

In these regression analyses, and all subsequent analyses for which water [As] was a significant predictor, the amount of As consumed from water was also identified as a significant predictor. However, these two variables were highly collinear and therefore only one could be used in the model. In each case, water [As] allowed for a higher model adjusted R^2 value and greater homoscedasticity of residuals, so it was placed in the models instead of water As consumption.

3.4.4 Factors Associated with Urinary Inorganic (As(III) and As(V)) Arsenic Concentration (Table 3.9 & Table 3.11)

Similar to total urinary [As], the most significant predictor for both urinary arsenite and arsenate concentrations was the participant's drinking water [As]. In the urinary [As(III)] regression analysis, using the lowest quartile (category 1) of water [As] as the reference level, the second quartile water [As] range was found not to differ significantly, while both the third and fourth quartiles of water [As] were found to be significantly different in their prediction of urinary [As(III)]. Participants whose water sample was in the third quartile water [As] range were predicted to have an increase of 1.9 times (95% CI: 1.2 – 2.8) the [As(III)] in urine, and those with a water sample in the fourth quartile water [As] group had a predicted increase of 3.2 times (95% CI: 2.1 – 5.0) the [As(III)] in urine, as compared to the reference group. Similar results were found for the logistic regression analysis of urinary [As(III)], with the third and fourth quartiles of water [As] increasing the odds of a samples having an As(III) concentration of >MDL by 14.3 (95% CI: 3.9 – 52.7) and 12.6 (95% CI: 2.9 – 54.4) times, respectively, compared to the first quartile baseline. In addition to water [As], the amount of

seafood consumed also influenced the concentration of As(III) in urine, though this association was only found in the linear regression analysis. An increase in seafood consumption from the 25th to the 75th percentile (0 – 4 oz. or 113.2 g) in the three days prior to urine sampling predicted a urinary [As(III)] increase of 1.4 times (95% CI: 1.2 – 1.6).

In the urinary [As(V)] linear regression analysis , participants with water samples in the second and third quartiles for [As] were found to not have significantly increased predicted concentrations of As(V) in urine as compared to those whose water was in the first quartile. However, participants with a water sample in the fourth quartile were predicted to have an increase of 2.5 times (95% CI: 1.2 – 5.4) the [As(V)] in urine, as compared to participants whose water sample was in the first quartile of water [As].

In the logistic regression analyses, water [As] was a significant predictor of urinary [As(V)] as a quartile, categorical variable. Water [As] in the third and fourth quartiles were associated with significantly higher urinary [As(V)], by 5.7 (95% CI: 1.7 – 18.8) and 8.7 (95% CI: 2.8 – 27.0) times, respectively, compared to baseline. For the linear regression analysis of urinary [As(V)], participants who consumed chicken in the three days prior to urine sample collection had a predicted urinary [As(V)] of 0.5 times (95% CI: 0.3 – 0.7) those who did not eat any chicken. Logistic regression analysis found chicken consumption to increase the odds of having a urinary [As(V)] of greater than the MDL by 2.0 times (95% CI: 1.0 – 4.1) ($p = 0.062$).

3.4.5 Factors Associated with Methylated (MMA(V) and DMA(V)) Urinary Arsenic Metabolite Concentration (Table 3.9 & Table 3.11)

Both urinary MMA(V) and DMA(V) concentrations were associated with the pH of a participant's main water source, according to linear regression analyses, with an increase in water pH from the 25th to the 75th percentile accounting for an increase of 1.3 times (95% CI: 1.1 – 1.6) and 1.3 times (95% CI: 1.1 – 1.5) the concentration of urinary MMA(V) and DMA(V), respectively. Logistic regression analysis did not find water pH to be a significant predictor of urinary [MMA(V)].

The amount of seafood consumed in the 3 days prior to urine collection was found to be a significant predictor of urinary [DMA(V)], with an increase in seafood consumption from the 25th to the 75th percentile (0 – 4 oz. or 113.4 g) accounting for an estimated increase of 1.4 times (95% CI: 1.3 – 1.5) the concentration of DMA(V) in urine.

A significant interaction was found for both urinary MMA(V) and DMA(V) regression models, in both linear and logistic regression results, between location and water [As], and therefore their coefficients are difficult to interpret. Figures 3.1 and 3.2 demonstrate these interaction effects for the linear regression analyses. In the linear regression analysis, it was shown that when taking water pH into account, participants in Hubbards were predicted to have slightly but significantly higher concentrations of MMA(V) in urine than participants from Fall River in the third quartile water [As] range ($p = 0.019$) and in the fourth quartile water [As] range ($p = 0.007$), by 30% and 57%, respectively. Logistic regression analysis also found that Hubbards participants had significantly ($p = 0.015$) higher urinary

MMA(V) concentrations in the highest water [As] category, by 61%, than those from Fall River. Similarly, participants from Hubbards were predicted to have significantly higher concentrations of urinary DMA(V) than participants from Fall River, by 55%, in the category with the highest 75% of water [As] ($p= 0.003$), when taking water pH and seafood consumption into account.

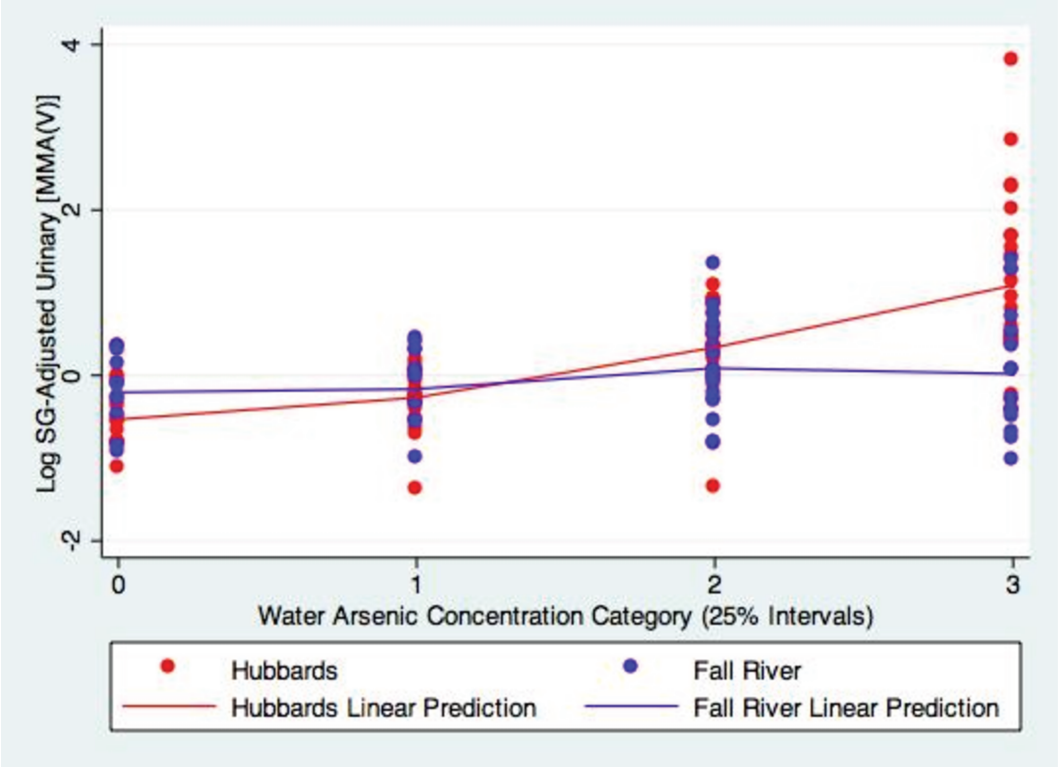

Figure 3.1 Concentration of urinary MMA(V) (μg/L) based on water arsenic concentration, categorized by location (red = Hubbards, blue = Fall River) (n=119)

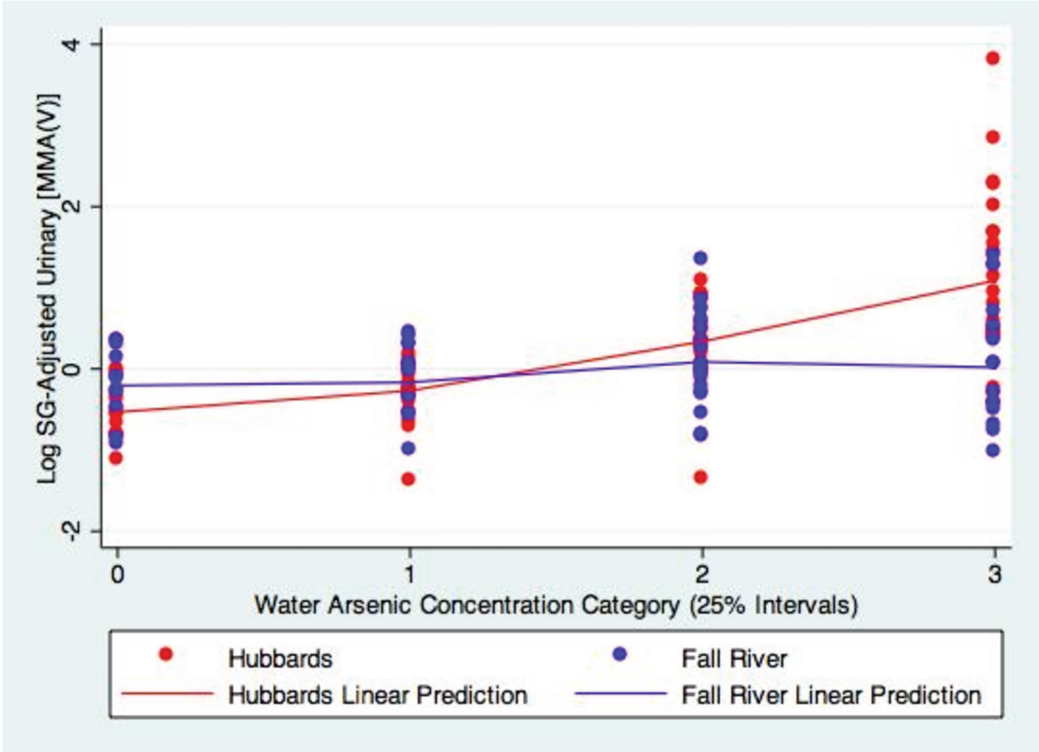

Figure 3.2 Concentration of urinary DMA(V) (μg/L) based on water arsenic concentration, categorized by location (red = Hubbards, blue = Fall River) (n=141)

3.4.6 Factors Associated with Urinary Arsenobetaine Concentrations (Table 3.9)

Urinary [AsB] was associated with reporting of seafood consumption in the last 3 days, with an increase of 4.0 times (95% CI: 2.5 – 6.4) the concentration of urinary AsB for participants who ate seafood compared to those who did not As well, the age of a participant predicted the [AsB] in urine, increasing the expected value by 1.02 times (95% CI: 1.01 – 1.04) for participants who were over 50 years of age, compared to younger participants.

3.4.7 Total and Total Minus Arsenobetaine (TMA) Urinary Arsenic Concentration in relation to the Nova Scotia Department of Environment (NS DOE) Maximum Contaminant Limit for Arsenic in water (Table 3.10)

After controlling for household income and As consumed from seafood, chicken and rice, linear regression analysis found that total urinary [As] was not significantly associated with water [As] of between 10 – 50 μg/L, as compared to <10 μg/L. However, a water [As] of >50 μg/L predicted a total urinary [As] that was 2.2 times (95% CIL 1.2 – 3.7) higher than for water [As] of <10 μg/L. As well, water [As] of between 20 – 50 μg/L and >50 μg/L were significantly associated with concentrations of urinary TMA that were 1.8 times (95% CI: 1.2 – 2.8) and 2.9 times (95% CI: 1.4 – 5.9) higher than the <10 μg/L category.

3.5 Discussion

Water [As] was a significant predictor of the concentration of all urinary As metabolites with the exception of AsB, which is derived mainly from seafood. Age was positively associated with total urinary [As] and urinary [AsB], while an increasing water pH predicted increases in urinary [TMA], [MMA(V)], and [DMA(V)]. Seafood consumption was associated with higher concentrations of total urinary [As], [TMA], [As(III)], [DMA(V)], and [AsB], while chicken consumption had mixed effects on the concentration of urinary As(V).

Similar to other studies, the concentration of As in a person's primary water source was found to be one of the most influential and consistent predictors of every urinary As metabolite investigated, with the exception of arsenobetaine (Table 3.9). Ingested As, whether from food or water, is absorbed through the gastrointestinal tract and accumulates in the liver. While a small amount of metabolism may take place in the bloodstream (mainly reduction of As(III)) (Marafante et al., 1987), the majority occurs in the liver. In the liver, inorganic forms of As are reduced and methylated to organic forms of As, including MMA(V) and DMA(V). These metabolites are then returned to the bloodstream, and are filtered into the urine via the kidneys for excretion (National Research Council, 2001). Interestingly, although previous research has suggested that accounting for water consumption is key to accurately reflecting exposure, our work has shown that the estimated amount of As consumed from water was not as highly associated with the total or speciated concentrations of As in urine as the concentration of As in water. Similar findings have been noted by other researchers (Karagas et al.,

2000; Rivera-Nunez et al., 2011). This finding may suggest that efforts to collect detailed information about people's drinking habits may not be a good use of time and resources, when the concentration of As in water is known.

Seafood consumption was consistently significantly associated with the concentration of all urine As species, with the exception of As(V) and MMA(V) (Table 3.9). This suggests that As consumed from seafood (arsenosugars, arsenolipids, arsenocholine) may be metabolized to As(III) in humans. While there has been evidence of seafood-derived As being metabolized to DMA(V) (Choi et al., 2010; Francesconi, Tanggaar, McKenzie, & Goessler, 2002), there have been no published accounts of seafood-derived As being metabolized to As(III). Originally considered to not be metabolized in humans, interest into the toxicity and metabolism of arsenosugars, arsenolipids, and arsenocholine has increased in the last decade, and research has consistently uncovered more metabolites associated with these arsenic products in humans (Francesconi et al., 2002; Navas-Acien et al., 2011; Schmeisser, Goessler & Francesconi, 2006; Schmeisser, et al., 2006). Research is ongoing, but it has been established that AsB is excreted unchanged in urine and is non-toxic, however it has also been shown that excreted amounts of AsB can be larger than the estimated amount consumed, suggesting that AsB may be *formed* in the body (Molin, Ulven, et al., 2012). Also, arsenosugars and arsenolipids, many of which have yet to be accurately chemically characterized (Raml et al., 2005), have been found to result in metabolites that are capable of damaging DNA (Andrewes et al., 2004). While the vast majority of As in seafood is in the more complex organic AsB, both sugar and lipid forms, small amounts of iAs

may still be present. As posited by Molin et al., As(III) in urine may be due either to biotransformation of larger As molecules, or a result of the iAs being present in the food being consumed (Molin, Ydersbond, et al., 2012). Due to the cross-sectional nature of this study, and the limitation of As speciation data of consumed foods, it is not possible to confirm that the relationship between increased As(III) in urine with increase seafood consumption indicates biotransformation of complex organic As molecules to As(III).

Participants older than 50 years of age were only predicted to have higher concentrations of total urinary As and AsB, although older participants did have slightly higher concentrations of the other urinary metabolites as well (Table 3.9). This may be partly due to the fact that participants over 50 years old ate slightly more seafood (mean: 3.1 oz. vs. 2.1 oz., not significantly different), although amount of seafood consumption was in the final total [As] model and therefore controlled for, so even among people eating the same amount of seafood, older people were associated with higher urinary total [As] than younger people. Also, older participants had lower median water [As] (1.3 μg/L vs. 8.6 μg/L, not statistically different) and lower median water As consumption values (2.8 μg vs. 9.0 μg, not statistically different) than younger participants, making this significant association difficult to interpret. While it has been documented in the literature that age can be associated with varying As metabolism, there have been no accounts of age affecting the concentration of total As excreted in urine, only that of specific As metabolites, as methylation capacity changes with age (Hopenhayn-Rich, Biggs, Kalman, Moore, & Smith, 1996; Lindberg et al., 2008; Vahter, 2000). As

well, the observed differences reported between age and urinary As metabolites are most often between adults and young children (Concha, Nermell, & Vahter, 1998; Lindberg et al., 2008), whereas the differences in the present study are between people younger or older than 50 years. Karagas et al. (2000) found that there was a decreasing relationship between participant age and toenail [As] for people with a water [As] of <1 μg/L ($p<0.001$) and >1 μg/L ($p = 0.01$), which may suggest that older people may have more As available in their body to be excreted via urine because less is being incorporated into their toenails, and perhaps other keratin-rich tissues. This effect of lower As concentrations in toenails with increasing age was not found in our dataset, nor was there evidence of this effect in fingernails or hair (see Chapter 4). Failing any other logical conclusions, it is possible that the observed relationship in these models between age category and total urinary [As] and AsB may be spurious, and no actual relationship exists in reality.

Consumption of chicken in the 3 days prior to urine collection was shown to increase the predicted concentration of urinary As(V) in logistic regression analyses (Table 3.11), but predicted lower urinary [As(V)] in linear regression analysis (Table 3.9). This finding suggests that chicken consumption may affect the concentration of As(V) in urine only at very low levels (near the detection limit of 0.709 μg/L), or that perhaps the low sample size in the linear regression analysis is producing unreliable estimations. In this case, the logistic regression analysis, with over 3 times as many observations, is likely to be more representative of the true association between chicken consumption and urinary [As(V)], since there is no

known reason that chicken consumption should decrease the amount of As(V) in urine. Assuming that the logistic regression coefficients are more reliable, this finding is still somewhat unexpected, since the majority of As in chicken is in the organic form (Schoof et al., 1999; Lijun Yang et al., 2011). Because As metabolism is, for the most part, a one-way pathway, organic As species do not normally get metabolized back to inorganic forms. One published study found a significant positive relationship between chicken consumption and total urinary [As] (Rivera-Nunez et al., 2011), and another suggested that As consumed from chicken could potentially amount to significant exposure (Lasky, Sun, Kadry, & Hoffman, 2004). In that study, analyses were not performed to determine which factors were associated with inorganic As in urine, but analyses found that chicken consumption was not associated with the concentration of urinary DMA(V) or AsB, suggesting that the observed increase in total urinary [As] came by way of inorganic As or MMA species. The chicken As values used in the present study, obtained from Schoof et al. (1999), were American values and may not fully represent poultry products in Canada, which could possibly contain different amounts of inorganic As. While inorganic As is no longer added to poultry feed by the three main poultry veterinarians in Nova Scotia, use of As in poultry feed is legal (Government of Canada, 2012) and its use is not overseen by any governing body in the province, and therefore it could still be in use in some farming practices (Dr. Randall Bishop, Cornwallis Veterinarians, Ltd., personal communication, January 22, 2013). Regardless of As use on poultry farms in Nova Scotia, poultry products available at grocery stores in the province can be products of other regions in Canada, the

United States, or other countries. Therefore, it is difficult to estimate the amount of As in the chicken consumed by participants, without directly testing the product. In the present study, the increase in predicted urinary [As(V)] was associated with the reported consumption of chicken, but not with any specific assumed As concentration, and therefore the observed relationship remains valid, regardless of the actual concentration of As in chicken.

Both urinary MMA(V) and DMA(V) had a significant positive relationship with the water pH level of a participant's primary drinking water source, although this association was found only in the linear regression analysis for MMA(V) (Table 3.9), and not in the logistic regression (Table 3.11). At higher pH levels, As that is adsorbed to soils is more hydrologically available, and previous studies have shown that a high pH is associated with higher [As] in groundwater in reducing environments (Canadian Council of Ministers of the Environment, 1997; Kinniburgh & Smedley, 2002). In this study, there was a strong increasing relationship between water [As] and water pH level in Hubbards, but not in Fall River (p-values <0.009 and 0.099, respectively). This effect was investigated by creating an interaction variable between pH and location. Although this interaction term was significant in a model with only these two predictors, it became non-significant when water [As] was added to the model. The interaction term between location and water [As], however, was significant in both MMA(V) and DMA(V) models. In each case, participants in Hubbards were predicted to have a higher urinary [As] (while controlling for water pH) at high water [As] compared to participants from Fall River at the same high water [As], but this association, while

statistically significant, was quite small for MMA(V). The coefficient of the interaction for urinary DMA(V) showed a larger difference between locations at the highest water [As] category. These findings suggest that the association between water [As], and therefore As consumption from water, has a greater effect on urinary MMA(V) and DMA(V) concentration for participants in Hubbards than those in Fall River, with the largest differences found at the highest water As concentrations. It is not known why such different associations between water [As], and [MMA(V)] and (DMA(V)] are observed, but it may due in part to the fact that Fall River is known to have elevated levels of As in soil (and perhaps air), while Hubbards does not. Fall River is located in an old gold-mining region and is known to have elevated concentrations of As in soil (Meunier et al., 2010), so participants from this area may have additional exposures to As that have not been accounted for (via soil or air through gardening or other outdoor activities), though only water samples were collected for analysis of [As], not soil or air. Hubbards, on the other hand, has elevated concentrations of As in water only, not in soil, and therefore our estimation of As ingestion for participants in Hubbards may be more accurate than those for Fall River, resulting in a stronger relationship between urinary As species and ground water [As]. The two sample locations were purposefully chosen for this study, based on known instances of elevated As in groundwater, to maximize the number of well water samples that were greater than 10 μg/L. The participants in the communities were not chosen randomly and therefore interpretation of any differences found between locations is cautioned.

An increasing water pH level was also associated with higher urinary [TMA] (Table 3.9). Once again, it is possible that this association is a reflection of the relationship between high water [As] and high pH levels, as discussed above, and that the majority of the TMA concentration is made up of MMA(V) and DMA(V), while As(III) and As(V) contribute much less. Removing the pH variable from the regression analyses for MMA(V), DMA(V), and TMA resulted in lower adjusted-R^2 and pseudo-R^2 values, but coefficients and p-values of other predictor variables did not change drastically (<25%), suggesting that water pH contributes to the urinary As metabolite concentrations independently of water [As] and location.

There have been no published reports that suggest water pH affects the metabolism of As in the body, and therefore water pH should have no direct effect on the concentration of individual urinary As metabolites. While pH may have an indirect effect on the total amount of As in urine, by increasing the availability of As in groundwater via soil, it had no significant association with total urinary [As].

Normally, the percentage of total As in a urine sample that is inorganic in nature is 10-30%, although the published literature has not broken inorganic As into its constituents (Vahter, 1999). As presented in Table 3.5, our research has shown that arsenite and arsenate made up approximately 3.2% and 4% of total urinary As in an average person (median: 2.7%, SD: 3.6%; and median 0%, SD: 9%). After removing, arithmetically, the As in urine that came mainly from consuming seafood, arsenobetaine (TMA), As(III) and As(V) comprised approximately 5.0% and 7%, respectively, of TMA in a typical participant (median:

4.8%, SD: 5.1% and 7%, SD: 14%, respectively). With urinary arsenite and arsenate combined, the percentage total inorganic As in an average participant's total urinary As was 7% (median: 3%, SD: 11%), and when subtracting urinary AsB from the total urinary As, TMA, the mean percentage total inorganic As in urine was 11% (median: 7%, SD: 14%). This population appears to be at the low end of the 10-30% inorganic urinary As range, which could represent a high overall efficiency in methylating inorganic As to methylated arsenicals. However, this population also has somewhat lower than average percentages of MMA(V) and DMA(V) in urine (especially when not considering the amount of As in the AsB form), suggesting that much of the total urinary [As] is made up of AsB and other, non-identified As compounds (Table 3.5).

In reference to the analyses in Table 3.10, these data supports the decision made by the Nova Scotia Department Of Environment (NS DOE) to lower the maximum allowable [As] in drinking water from 50 μg/L to 10 μg/L. It was found that, after controlling for household income and As consumed from food, water [As] of greater than 20 μg/L, and to a lesser significance between 10 – 20 μg/L, were highly significantly predictive of an increased concentration of As in urine. Perhaps more relevant is finding that water [As] of between 20 – 50 μg/L were found to be positively associated with urinary [TMA], which is likely a better representation of that potential risk than total urinary [As], since that value includes potentially large amounts of AsB, which is non-toxic. It is recommended that future studies and policies should be conducted and formed based on a

urinary As measurement that does not include AsB, to more accurately reflect the amount of potentially toxic As in the body.

In both linear and logistic regression analyses, potential explanatory variables related to selenium (selenium concentration in water and urine) were investigated as potential confounders, due to the known biological interactions between As and selenium (Gailer, 2007). No confounding effects of selenium were observed in any regression models ($p>0.20$), and therefore these potential confounding variables were not included in final models. As discussed previously (Section 2.5), linear and logistic regression models were constructed using random effects variables that accounted for clustering at the household and location levels. In these models, the random effects portion of the variance observed in the outcome variables did not contributed meaningfully to the total observed variation. Therefore, the simpler models with only fixed effect variables were presented and discussed. Fixed effects models were fitted using robust standard errors, to incorporate some of the minimal effect that clustering may have had on this dataset.

While efforts were made to ensure the selected participants and collected information were unbiased, the results of this study should be considered in light of several potential limitations. Participants with little advanced warning of sample collection (<48 hours) were often unable to reliably recall what they had eaten or drank in the last 3 days, whereas participants with ample warning were able to make mental or physical notes on what they had eaten. Due to poor recall when participants were asked about the amount and type of seafood consumed,

assigning individual values of [As] for individual types of seafood consumed was not feasible. In this study, a single value for As content in seafood was used, regardless of the actual seafood type, which is known to vary between species. This measurement or misclassification error may have led to an underestimation or overestimation of specific foods (or water) that were consumed over the last 3 days, which would influence the association between urinary As metabolites and what was reported as As consumption. Altered methods of data collection for food consumption in future studies may be beneficial. Ingested water sources from outside the home, which were not tested for [As], could also confound our results.

For ethical reasons, when individuals were contacted to take part in the study, they were informed of their [As] in their home tap water from the screening process. For participants whose water was above the MCL of 10 μg/L, an adjustment of normal drinking habits may have occurred in the time between recruitment and subsequent water and urine sample collection for the main study. This could have misrepresented the amount of water consumed on a daily basis before the participant was aware of their water [As]. This may be more important, however, when comparing longer-term biomarkers such as hair and nails to the amount of As consumed from water in the last 2 days, which was taken to be representative of "normal" exposure for each individual.

In terms of selection biases, in order to collect as much information as possible from individuals whose recruitment water sample contained greater than 10 μg/L of As, each household in this category was contacted. In contrast, only a fraction of participants in the <10 μg/L of As category were contacted, albeit by

random selection. The different techniques of participant selection may have introduced some selection bias into the study, though the bias, if in fact present, is expected to be small with a tendency towards the null.

Although the questionnaire and sampling techniques used were not ideal, we were still able to determine logical factors associated with urinary As metabolites.

3.6 Conclusion

The concentration of As in a person's primary water source was found to be one of the most influential and consistent predictors of every urinary As metabolite investigated, with the exception of arsenobetaine. Seafood consumption was also consistently significantly associated with the concentration of all urine As species, with the exception of As(V) and MMA(V). This suggests that some types of As consumed from seafood (arsenosugars, arsenolipids, arsenocholine) may be metabolized to toxic As(III) in humans, though this result requires confirmation. Participants older than 50 years of age were predicted to have higher concentrations of total urinary As and AsB only, and participants who lived in Fall River were predicted to have higher concentrations of urinary DMA(V) and MMA(V), with an interesting interaction between water [As] and location for the two metabolites. A higher water pH was associated with a higher urinary DMA(V), MMA(V), and TMA concentration.

With many parts of Nova Scotia having high total [As] in groundwater, it is important for residents to be aware of the concentration of As in their well water

and to be aware of the amount of seafood they are eating, particularly if they are living in areas known to have As in the groundwater. More research in the field of seafood-derived arsenic metabolism and other As species in humans is required to determine if any seafood derived As compounds are potential health threats for humans.

3.7 References

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4 Factors Associated with Human Hair and Nail Arsenic Concentrations

4.1 Abstract

Hair, fingernails, and toenails are widely used biomarkers of arsenic (As) exposure in humans. This study focuses on the relationship among demographic, lifestyle, and consumption factors and levels of As found in each of these biological samples.

In 2010, 179 participants from two locations in Nova Scotia, Canada, were chosen as the sample population to include an exposed group whose main water source contained at least 10 μg/L As, the current maximum allowable concentration (MAC) limit for As in drinking water, along with a suitable control group. Participants completed a questionnaire to collect water and food consumption information, as well as demographic and lifestyle information. A water sample and biological samples (hair, fingernails, toenails, and urine) were collected. Samples were analyzed for total [As] using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Water [As] ranged from less than the minimum detection limit (MDL) of 0.017 μg/L to 309.33 μg/L; hair [As]ranged from less than the MDL of 0.068 μg/g to 2.32 μg/g; fingernail and toenail [As] ranged from less than the nail MDL of 0.153 μg/g to 4.10 μg/g and 13.18 μg/g, respectively.

Multiple logistic and linear regression analyses found two predictive factors to be consistently positively associated with the [As] found in hair, fingernails, and toenails. A high [As] in a person's main source of drinking water (>50 μg/L for hair, >10 μg/L for nails) increased the odds of having a detectable [As] in hair,

fingernails, and toenails by 7.5 (95% CI: 2.1 – 27.0), 3.3 (95% CI: 1.2 – 9.4), and 6.8 (95% CI: 2.8 – 16.8) times, respectively ($p < 0.05$). A high concentration of selenium (Se) in each respective biological sample (top 25% of Se concentrations in each sample) increased the odds of having a detectable [As] in hair, fingernails, and toenails, by 5.7 (95% CI: 2.1 – 15.2), 25.9 (95% CI: 6.1 – 109.5), and 15.1 (95% CI: 3.5 – 65.4) times, respectively ($p< 0.001$). In addition to these factors, seafood consumption was a positive predictor of fingernail [As. Further research is needed into the metabolic relationship between As and Se and their mutual deposition in these tissues, and the metabolism of seafood-derived As.

4.2 Introduction

Assessing the degree to which someone has been exposed to As can be achieved in several ways. The most obvious and common method is to analyze a person's household drinking water for its [As], which can be accomplished by use of an inductively coupled plasma mass spectrometer (ICP-MS) (United States Environmental Protection Agency, 1994). After determining the [As] in a person's water supply, investigation into the amount of this water (and other liquids mixed with this water, or foods cooked or processed with this water) that is consumed will estimate an average daily consumption of As from a personal water source. However, this method is not able to capture any exposure to As that may occur outside of the home, unless other water sources outside the home are tested for As as well. Arsenic consumed from foods will also not be included in this exposure estimate, nor will any dermal or aerosol As exposure. Therefore, assessing an As

biomarker, such as urine, nails, or hair, may be more representative of total As exposure than any of these estimates of what is taken into the body.

When inorganic As (iAs) is taken into the body, it is metabolized in the body to organic forms of As, mainly monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)). Typically, the body excretes As primarily through urine, with an average metabolic profile in urine of approximately: 10-30% inorganic As (arsenite, As(III), and arsenate, As(V)), 10-20% MMA(V), and 60-70% DMA(V) (Vahter, 1999). However, As is also excreted through various other pathways, such as sweat and sebaceous glands, hair, fingernails, and toenails (Chen, Amarasiriwardena, & Christiani, 1999; Hindmarsh, 2002). The examination of factors associated with urine As biomonitoring has been reported elsewhere (see chapter 3), therefore, in this paper, we will focus on nails and hair.

Due to a strong affinity for sulfhydryl groups, As is easily incorporated into keratin proteins, a major component of human hair and nails. Mandal et al. (2003) used high performance liquid chromatography – inductively coupled argon plasma mass spectrometry (HPLC-ICP-MS) to speciate As in hair samples, finding individual species that contributed the following percentages to total As concentration: As(III) 60.9%, As(V) 33.2%, MMA(V) 2.2% and DMA(V) 3.6% (Mandal, Ogra & Suzuki, 2003). With an approximate growth rate of 10 mm/month (approximately 0.33 mm/day) (Harkey, 1993), hair has been, for several decades, used as a biomarker of exposure for both long- and short-term exposure studies for As, as well as other metals and compounds (Gault et al., 2008; Mosaferi et al., 2005;

Muikku, Puhakainen, Heikkinen, et al., 2009; Ndiokwere, 1985). Although hair is used extensively to gauge exposure to As over different time periods, there are suggestions that it may not be as reliable as some other biological samples (i.e. blood, urine, and nails). Sample length, sample collection site, As distribution within the sample (both length-wise and width-wise), and external contamination are several of the issues surrounding the use of hair as a biological monitoring tissue for As exposure. Several published articles have been written to express the use of caution when attempting to interpret [As] in hair samples (Cornelis, 1973; Hindmarsh, 2002; Smith, 1976), however its use continues to be widespread.

In the same metabolic process as in hair As deposition, As is readily incorporated into keratin proteins of nails. Mandal et al. (2003) used HPLC-ICP-MS to speciate As in fingernail samples and found that individual As species contributed the following percentages to total As concentration: As(III) 58.6%, As(V) 21.5%, MMA(V) 7.7%, DMA(III) 9.2%, DMA(V) 3.0% (Mandal et al., 2003). Human fingernails have an approximate growth rate of 0.1 mm/day, resulting in complete fingernail replacement approximately every 6 months, whereas toenails grow more slowly, at a rate of between 0.03 – 0.05 mm/day, requiring between 12 – 18 months for complete replacement of an entire nail (Fleckman, 1985). Given these growth rates, fingernails and toenails can offer a more historical biomarker of As exposure (particularly in those with short hair) than blood, urine or hair, which have As-clearing times of hours, days, and weeks, respectively (Buchet, Lauwerys & Roels, 1981; Harkey, 1993; Mealey, Brownell & Sweet, 1959).

However, as with hair samples, arsenic in water, soil, air and dust are all possible sources of external contamination of As in nails, which have been shown to absorb As from these external sources. Washing nail samples, for which there are many non-standardized procedures used in various chemical and epidemiological studies, has been found to remove large amounts of exogenous As deposits, but not all (Chen, Amarasiriwardena & Christiani, 1999). For this reason, toenails are often preferred as biomarkers of As exposure over fingernails, as they are less likely to be contaminated by exogenous sources (Marchiset-Ferlay, Savanovitch, & Sauvant-Rochat, 2012), due to the wearing of shoes and socks. As well, assessment of recent exposure to As is not possible with nails because nail tip As deposition is a function of the As exposure at the time it was produced, and we cannot easily obtain samples of recently produced nail.

As a biomarker, the [As] in hair and nails is a reflection of the amount of As that has successfully entered the individual (DeCaprio, 1997; Paustenbach, 2000). An [As] of 1.0 μg/g in hair and nails has been suggested as the threshold for "normal" background [As] in an unexposed population (Agency for Toxic Substances and Disease Registry (ATSDR), 2007; Choucair & Ajax, 1988; Hindmarsh, 2002). Concentrations of As in hair in individuals from areas with chronic As exposure have been found in the range of 0.1 to 8.0 μg/g (Gault et al., 2008; Mandal et al., 2003; Mosaferi et al., 2005). Similarly, fingernail and toenail As concentrations from people in areas of high As exposure have been reported to range from 0.2 to 7.3 μg/g and from 0.2 to 21.7 μg/g, respectively (Hinwood et al., 2003; Karagas et al., 1996; Kile et al., 2007).

As outlined in a review by Marchiset-Ferlay, hair, fingernails, and toenails are often used as biological markers of As exposure, however there is no clear evidence on which factors affect [As] in these tissues (Marchiset-Ferlay et al., 2012). In an effort to resolve these issues, the objective of this study was to investigate what factors may influence the [As] in each of these respective biological samples. To this end, relationships among various social, demographic, lifestyle factors, water and food consumption factors, and the measured [As] in hair, fingernail, and toenail samples were investigated using multivariable logistic and linear regression analyses. Information regarding how the [As] in these biological samples may fluctuate depending on these factors may assist in the appropriate design and implementation of future As biomonitoring programs involving hair and nails.

4.3 Materials and Methods

4.3.1 Screening Procedure to Identify a Sampling Frame and Study Groups

See section 2.3.1 of this thesis for methods pertaining to the identification and selection of the sampling frame and study groups, respectively.

4.3.2 Questionnaire Administration, Sample Collection, and Laboratory Analysis

In July of 2010, during a face-to-face interview, study participants completed a questionnaire designed to collect key demographic and personal information related to As exposure, such as: bathing frequency, use of hair dye, length of time spent in current residence, water filtration information, dietary

habits, water consumption habits, and others. For dietary considerations, participants reported the amount of certain types of foods that they consumed in the 3 days prior to the interview (especially chicken, rice, and seafood, as these may contain higher concentrations of As relative to other foods). For water consumption, participants reported all home tap water consumed during the 2 days prior to the interview.

Following the questionnaire, hair and nail samples were collected from all participants, when possible. Hair samples were collected from the nape of the neck, in a quantity that was as close to the width of a pencil as possible. Hair samples were collected using stainless steel, commercially available scissors, and were sterilized in alcohol between each participant. Nail samples were collected from each finger and toe (as available) using commercially available stainless steel nail clippers, which were disinfected with alcohol wipes between each participant. Additionally, a second water sample was collected during the home visit to obtain an accurate reflection of total and speciated water [As].

After collection, all samples were sent to Acadia University in Wolfville, Nova Scotia. Samples were analyzed for total [As] by ICP-MS and water and urine samples were also analyzed using HPLC-ICP-MS to determine the concentration of the different As species present in each of these samples. The minimum detection limit of the ICP-MS for As concentrations was 0.017 μg/L for water, 0.068 μg/g for hair, and 0.153 μg/g for nail samples. As well, because of known metabolic interactions between As and selenium (Se) (Levander, 1977), total [Se] was also

determined in all samples by ICP-MS. Due to budgetary constraints, only the total [As] and [Se] of hair and nails was quantified, and not individual species.

4.3.3 Descriptive Statistics

For the purpose of comparing groups for statistical analyses, participants were separated into two categories based on their degree of As exposure. Participants whose water contained less than 10 μg/L were placed in the "non-exposed" category, and all others, with water [As] of ≥ 10 μg/L, were placed in the "exposed" category.

To investigate any possible differences in hair and nail [As] between location, gender or exposure groups, permutation analyses were performed (Baglivo, 2005). Permutation analyses were used to investigate statistically significant differences between group medians (as hair and nail As concentrations were strongly right-skewed), by running 10,000 iterations of re-sampling with replacement, and comparing the actual difference between group medians with that of the permutated difference between group medians. This is a preferred method of determining statistically significant group differences in non-normally distributed data as a preliminary step prior to multivariable modeling.

4.3.4 Analytical Statistics

Hair, toenail, and fingernail [As] were categorized as either "below MDL" if less than the minimum detection limit, or "above MDL" if greater than the minimum detection limit (see section 2.3.3 for a discussion of MDL calculations). The dichotomized hair, toenail, and fingernail As concentration variables were

used in logistic regression models (as three separate outcome variables) to determine associations between these outcomes and social, demographic, lifestyle and consumption factors.

A second set of analyses investigated relationships among hair and nail [As] and demographic, consumption, and other questionnaire information through multivariable linear regression analysis (as three separate outcome variables). With a large number of samples with [As] <MDL, there was no transformation (log, square root, inverse, etc.) that was able to produce a suitable, normally distributed outcome variable. Therefore, linear regression analyses were performed on a sub-population of the sample population – only samples that had an [As] of >MDL. Among this sub-population, the outcome variables, hair, fingernail and toenail [As], were modeled using natural log transformed data, which normalized regression residuals. As previously discussed in Section 2.3.4 of this thesis, in these linear regression models, the outcome variables were natural-log transformed to obtain a normal distribution of values and model residuals. Continuous variables in log-transformed linear regression models will be interpreted as the percent change in the outcome variable that occurs when increasing the continuous independent variable value from the 25^{th} percentile to the 75^{th} percentile (i.e. the interquartile range). For categorical variables, all percent changes in the outcome variable will be interpreted with respect to the baseline category of the independent categorical variable.

Potential predictor variables for possible inclusion in the multivariable models were chosen first by conducting simple univariable associations with the

dependent variables, at a liberal p-value cut-off of 0.15. Whenever possible, predictor variables were included in models in continuous form, however, when a continuous variable was not significant (after attempting suitable transformations), it was transformed into a categorical variable and included in the model if it was significant. For example, for several analyses, water [As] was categorized based on maximum contaminant limits (MCL) for As in drinking water from various governments around the world: 10 μg/L, 20 μg/L, 50 μg/L, with the addition of < 10 μg/L and > 50 μg/L categories to encompass all data (Henke, 2009). The hair [Se] variable was also categorized as the lowest 50%, middle 25% and highest 25% of Se concentrations, based on the distribution of the data. Consumption of As and Se in water were calculated by multiplying the concentration of each element in a participant's home drinking water with the amount of water that a participant reported consuming over the previous 2 days.

Significant correlations between variables that were at least marginally significant in univariable analyses were investigated. Final models were constructed using manual step-wise inclusion to maximize adjusted-R^2 values, while preserving parsimony. When there were significant correlations between two variables, the variable that was most biologically plausible and produced the largest adjusted R^2 value in multivariable regressions was used. Variables in the final models required a $p < 0.05$ to remain as a model predictor.

For all regression analyses, possible confounding effects and interaction between significant model variables were considered. All linear regression models were tested for meeting the assumptions of linearity (visually: standardized

residuals vs. inverse normal curve; statistically: Shapiro-Wilk W test for normal data) and homoscedasticity (visually: residuals vs. fitted values plot; statistically: Cook-Weisberg test for heteroscedasticity). Logistic regression models were tested for goodness of fit (statistically: Pearson chi-squared and Hosmer-Lemeshow goodness-of-fit tests) and evaluated for influential covariate patterns (standardized residuals vs. covariate pattern plot; standardized residuals outside of ± 3), high leverage points (Pearson residual vs. leverage plot; >30% of mean value), and delta-betas (Pearson residuals vs. delta-beta plot).

All statistical analyses were performed using Stata 11 (StataCorp LP, College Station, Texas).

4.4 Results

4.4.1 Descriptive Statistics of Hair Arsenic Concentrations

In total, 150 hair samples were collected from the two study locations – 85 from Hubbards and 65 from Fall River. Hair samples had [As] ranging from less than the MDL of 0.068 μg/g to 2.3 μg/g. Of these, 90 samples were from "exposed" individuals whose water contained at least 10 μg/L. Of the 150 hair samples, 5 (3%) were above the recommended MCL of 1.0 μg/g – all from Hubbards. Of the hair samples above the MCL, 4 were from exposed individuals. Descriptive statistics of total [As] in hair are presented in Table 4.1 by location and exposure group, including columns for all samples and for only samples that have an [As] of greater than the MDL. Medians for "all samples", in all categories, were < MDL and therefore are not provided in Table 4.1. There were no significant differences

between locations when considering hair samples with an [As] greater than the MDL.

Table 4.1 Descriptive statistics of hair arsenic concentrations, by location and exposure

Category	n	n	Hair [As] Mean (95% CI)	Hair [As] Mean (95% CI)	Hair [As] Median (range)
	All samples	If >MDL	All samples	If >MDL	If >MDL
Total	150	49	0.15 (0.10 – 0.21)	0.40 (0.25 – 0.54)	0.20 (0.07 - 2.32)
Hubbards	85	31	0.21 (0.12 – 0.31)	0.53 (0.30 – 0.75)	0.31 (0.07 – 2.32)
Fall River	65	18	0.07 (0.034* – 0.09)	0.18 (0.13 – 0.23)	0.16 (0.07 – 0.43)
Unexposed	90	21	0.10 (0.034* – 0.15)	0.31 (0.11 – 0.52)	0.19 (0.07 - 2.15)
Exposed	60	28	0.23 (0.12 – 0.34)	0.46 (0.24 – 0.67)	0.23 (0.07 - 2.32)

Data in μg/g

*Data with an asterisk represent a value of one half of the minimum detection limit for hair (0.068 μg/g)

95% CI: 95% confidence interval, MDL: minimum detection limit

4.4.2 Analytical Statistical Analyses of Factors Associated with Hair Arsenic Concentration

From Pearson correlation coefficients calculated between water [As] and hair [As], participants from Hubbards had larger coefficients than from Fall River for all water categories (water [As] categories of <10, 10-20, 20-50, and >50 μg/L). For example, participants in Hubbards showed higher Pearson correlation coefficients between hair and water [As] than those in Fall River, with values of 0.6015 and 0.0695, respectively. Due to the differences observed in Pearson correlation coefficients between locations, the location variable was forced into all linear and logistic models, to control for any differences between locations. Table 4.2 reports univariable logistic regression analysis results for predictor variables that were at least moderately significant in the prediction of dichotomized hair [As]. For the logistic regression analyses, hair samples were dichotomized at the ICP-MS MDL of 0.068 μg/g of As; logistic regression analyses therefore reflect what variables were associated with a person having a hair [As] of less than or greater than the MDL. Only age, nail [As], and a number of urine and water As metabolite concentrations were at least marginally positively associated with hair [As], without considering confounding.

Table 4.2 Univariable logistic regression analyses results, with dichotomized hair arsenic concentration as the outcome, for variables with at least moderate significance (p < 0.15)

Variable	n	Variable Description	Hair [As] OR (95% CI)	p-value
Age (>50)	148	0 - <50, 1 >50	0.57 (0.28 - 1.17)	0.127
Age (category)				0.113
<39 years	16		Reference	
40-60 years	59		0.40 (0.13 - 1.23)	0.109
>60years	74		0.31 (0.10 - 0.93)	0.037
Nail [As]	130	Range: 0.077 - 13.2 μg/g	1.91 (1.00 - 3.66)	0.050
Urinary [As(III)]	130	Range: 0.355* - 19.7 μg/L	1.37 (1.04 - 1.81)	0.027
Urinary [MMA(V)]	129	Range: 0.355* - 45.3 μg/L	1.43 (1.07 - 1.91)	0.017
Urinary [DMA(V)]	130	Range: 0.355* - 217.5 μg/L	1.03 (1.00 - 1.06)	0.050
Urine % AsB	130	Range: 0 - 139.9	0.98 (0.97 - 0.99)	0.005
Urine % As(III)	130	Range: 0 - 22.5	1.14 (1.02 - 1.27)	0.016
Urine % MMA(V)	130	Range: 0 - 18.4	1.09 (1.00 - 1.18)	0.052
Urine % DMA(V)	130	Range: 0 - 95.7	1.03 (1.01 - 1.05)	0.004
Water As Intake	150	Range: 0 - 1,798.8 μg	1.01 (1.01 - 1.02)	0.002
Water [As]	150	Range: 0.0085* - 309.2 μg/L	1.02 (1.01 - 1.04)	0.004
Water [As(V)]	73	Range: 0.5 - 235.7 μg/L	1.02 (1.00 - 1.04)	0.087
Water [As] >10 μg/L	150	% >10 μg/L: 41%	2.89 (1.42 – 5.81)	0.003

*Data with an asterisk represents a value that is half of the minimum detection limit for that sample (MDLs: urine – 0.709 μg/L, nails – 0.153 μg/g, water – 0.017 μg/L)
95% CI: 95% confidence interval
As(III): arsenite, As(V): arsenate, AsB: arsenobetaine, MMA: monomethylarsonic acid, DMA: Dimethylarsinic acid
Urine %: Percentage of total urinary arsenic accounted for by a specific metabolite

Table 4.3 presents the final multivariable logistic regression model for the prediction of dichotomized hair [As]. All regression models met the assumptions of independence and linearity, and goodness of fit (Hosmer-Lemeshow $p = 0.748$).

Table 4.3 Multivariable logistic regression analysis for dichotomized hair arsenic concentration of greater than the MDL versus less than the MDL of 0.068 μg/g

Variable	OR (Robust St. Error)	95% CI	p-value
Hair [Se] Category			0.003*
1 - <0.233 μg/g	Referent		
2 - 0.233 - 0.424 μg/g	2.99 (1.46)	1.15 - 7.80	0.025
3 - >0.424 μg/g	5.72 (2.96)	2.08 - 15.77	0.001
Water [As] Category			0.006*
1 - <10 μg/L	Referent		
2 - 10 - 20 μg/L	2.04 (1.14)	0.68 - 6.11	0.204
3 - 20 - 50 μg/L	2.73 (1.31)	1.06 - 7.00	0.037
4 - >50 μg/L	7.43 (4.69)	2.16 - 25.60	0.001
Location	0.77 (0.31)	0.35 - 1.68	0.506
Water Se Intake Category			0.808*
1 - <0.05 μg	Referent		
2 - 0.02 - 0.12 μg	0.86 (0.51)	0.27 - 2.75	0.802
3 - 0.12 - 0.37 μg	0.89 (0.42)	0.35 - 2.27	0.803
4 - >0.37 μg	0.53 (0.35)	0.14 - 1.94	0.337
Constant	0.18 (0.08)	0.08 - 0.45	
n	147		
Pseudo-R^2	0.1532		

*Overall p-value for categorical variables
95% CI: 95% confidence interval, Se: Selenium
Location: 0 = Hubbards, 1 = Fall River

- Hair arsenic logistic model with logit coefficients:

ln[p(hair [As] >MDL)/1 – p(hair [As] >MDL)]: -1.7 + [1.1*(hair [As] categ. 2)] + [1.8*(hair [As] categ. 3)] + [0.7*(water [As] categ. 2)] + [1.0*(water [As] categ. 3)] + [2.0*(water [As] categ. 4)] + [-0.3*(location)] + [-0.2*(Se intake categ. 2)] + [-0.1*(Se intake categ. 3] + [-0.6*(Se intake categ. 4)]

In Table 4.3, the amount of As ingested from water and the [Se] in hair samples were significant predictors of having a hair [As] of greater than the MDL of the ICP-MS, when controlling for location. In this analysis, and subsequent linear and logistic analyses for the prediction of [As] in hair and nails, the amount of Se consumed from water was included to control for its potential confounding effects on the [Se] in these tissues. Because the amount of Se consumed from water was likely to contribute to the [Se] observed in hair and nails, controlling Se consumed in water allowed for the interpretation of [Se] in nails that was due to the relationship between As and Se, and not due to the amount of Se consumed. Both the location and Se intake variables were non-significant predictors of having a hair [As] of >MDL, and regression coefficients did not change significantly (<10%) with their addition. Selenium intake from water ranged from 0 – 48.4 μg. Participants whose primary source of drinking water was in the 20 – 50 μg/L and > 50 μg/L of As ranges had significantly higher odds of having a detectable [As] in their hair, of 2.7 times (95% CI: 1.1 – 700) and 7.4 times (95% CI: 2.2 – 25.6), respectively), as compared to participants in the < 10 μg/L category. Also, participants with hair [Se] in the 0.23 – 0.42 μg/g or > 0.42 μg/g categories had significantly higher odds, of 3.0 times (95% CI: 1.2 – 7.8) and 5.7 times (95% CI: 2.1 – 15.8), respectively, of having detectable levels of As in their hair as compared to participants with less than 0.23 μg/g of Se in their hair.

Table 4.4 presents the results of univariable linear regression analyses of variables that were at least moderately significant ($p < 0.15$) in the prediction of natural log transformed hair [As], for the sub-population with hair samples that

were greater than the MDL. Without considering confounding, using univariable regression analysis, hair [As] was at least marginally higher in participants who were older, male, lower income, less educated, did not use hair dye, and from Hubbards, who consumed seafood in the last 3 days. Additionally, univariable regression analyses found that hair [As] was at least marginally significantly positively associated with hair [Se], total As intake, and a number of urine and water As metabolite concentrations. Percentages of urinary arsenic metabolites are based on specific-gravity adjusted urinary arsenic metabolite concentrations. Due to the adjustment of urinary arsenic metabolites relative to urine dilution, and percent recovery by ICP-MS, specific urinary arsenic metabolites can, though rare, have percentages of greater than 100%. In this dataset, 139.9% was the maximum value found, with the next highest being 92.9%.

Table 4.4 Univariable linear regression analyses results, with natural log transformed hair arsenic concentration for samples with more than the MDL as the outcome, for variables with at least moderate significance (p < 0.15)

Variable	n	Variable Description	Coef. (95% CI)	p-value
Age (>50)	49	0 - <50 years, 1 >50 years	0.56 (0.02 - 1.11)	0.044
Age (years)	49	Range: 28 – 83	0.02 (0.004 - 0.042)	0.018
Age (category)				0.048
<39 years	9		Reference	
40-60 years	20		-0.01 (-0.76 - 0.74)	0.974
>60years	20		0.68 (-0.07 - 1.43)	0.074
Education				0.078
Primary	4		Reference	
Secondary	17		-1.23 (-2.27 - -0.19)	0.021
College	9		-1.37 (-2.49 - -0.24)	0.018
University	19		-1.31 (-2.34 - -0.29)	0.014
Gender	49	0 - Male, 1 - Female	-0.44 (-1.00 - 0.11)	0.114
Hair [Se]	49	Range: 0.1 - 39.1 μg/g	0.05 (0.01 - 0.09)	0.010
Hair Dye	49	Use hair dye: % yes - 27%	-0.51 (-1.10 - 0.08)	0.088
Income				0.106
<$30,000/year	5		Reference	
$30,000-$50,000/year	13		-0.22 (-1.24 - 0.81)	0.673
>$50K/year	28		-0.78 (-1.73 - 0.17)	0.104
Location	49	0 - Hubbards, 1 - Fall River	-0.69 (-1.24 - -0.14)	0.015
Seafood	49	Seafood consumed in last 3 days:% yes – 16%	0.47 (-0.08 - 1.03)	0.094

Coefficients presented in natural log format
95% CI: 95% confidence interval
MDL: Minimum detection limit, 0.068 μg/g for hair, Se: selenium

Table 4.4 Univariable linear regression analyses results, with natural log transformed hair arsenic concentration for samples with more than the MDL as an outcome, for variables with at least moderate significance (p < 0.15) (continued)

Variable	N	Variable Description	Hair [As] Coef. (95% CI)	p-value
Total As Intake	49	Range: 0.02 - 3148.30 μg	0.001 (-0.001 - 0.001)	0.145
Urinary [As(III)]	45	Range: 0.355* - 19.7 μg/L	0.07 (0.001 - 0.141)	0.046
Urinary [DMA(V)]	45	Range: 0.355* - 217.5 μg/L	0.01 (-0.002 - 0.013)	0.117
Urinary [TMA*]	44	Range: 3.5 - 293.1 μg/L	0.01 (-0.001 - 0.010)	0.106
Urine % As(III)	45	Range: 0 - 13.0	0.06 (-0.01 - 0.14)	0.104
Water [As]	49	Range: 0.0085* - 309.2 μg/L	0.01 (0.003 - 0.009)	< 0.001
Water [As(III)]	15	Range: 0.3 - 249.0 μg/L	0.01 (0.003 - 0.015)	0.008

Coefficients presented in natural log format

*Data with an asterisk represent a value of half of the minimum detection limit for that sample (MDLs: urine – 0.709 μg/L, water – 0.017 μg/L)

95% CI: 95% confidence interval

As(III): arsenite, As(V): arsenate, DMA: Dimethylarsinic acid, TMA: Total urinary arsenic minus arsenobetaine, Urine %As(III): Percentage of total urinary arsenic accounted for by As(III)

MDL: Minimum detection limit, 0.068 μg/g for hair

Table 4.5 reports the final linear regression model for predicting natural log transformed hair [As] in the sub-population with hair [As] of >MDL. Coefficients and confidence intervals are in natural log transformed format. All regression models met assumptions of independence, linearity and homoscedasticity (p-values > 0.15).

Table 4.5 Multivariable linear regression analysis, with natural log-transformed total hair arsenic concentration as the outcome, for 48 hair samples with an arsenic concentration of greater than the MDL of 0.068 μg/g

Variable	Coef. (Robust St. Error)	95% CI	p-value
Hair [Se]	0.06 (0.02)	0.02 - 0.10	0.006
Water [As] Category			0.044*
1 - <10 μg/L	Referent		
2 - 10 - 20 μg/L	0.19 (0.24)	-0.30 - 0.68	0.439
3 - 20 - 50 μg/L	0.03 (0.28)	-0.55 - 0.60	0.919
4 - >50 μg/L	1.25 (0.43)	0.37 - 2.13	0.006
Location	-0.32 (0.25)	-0.83 - 0.19	0.214
Water Se Intake Category			0.864*
1 - <0.05 μg	Referent		
2 - 0.02 - 0.12 μg	0.02 (0.28)	-0.55 - 0.60	0.932
3 - 0.12 - 0.37 μg	-0.23 (0.35)	-0.93 - 0.48	0.523
4 - >0.37 μg	0.06 (0.33)	-0.62 - 0.73	0.867
Constant	-1.71 (0.31)	-2.33 to -1.10	<0.001
n	48		
Adjusted-R^2	0.4530		

*Overall p-value for categorical variables
95% CI: 95% confidence interval
Negative coefficients become positive when transformed; Coefficients in natural log format
Location: Hubbards=0, Fall River=1

- Hair arsenic concentration model with back-transformed coefficients:
  Hair [As] = 0.2 + [1.1*(hair [Se])] + [1.2*(water [As] categ. 2)] + [1.0*(water [As] categ. 3)] + [3.5*(water [As] categ. 4)] + [0.7*(location)] + [1.0*(water Se intake categ. 2)] + [0.8*(water Se intake categ. 3)] + [1.1*(water Se intake categ. 4)] + error

Hair [Se] was positively associated with hair [As], with a predicted increase of 1.03 times (95% CI: 1.01 – 1.05) the concentration of As in hair for an increase in hair [Se] from the 25th to the 75h percentile (0.2 – 0.7 μg/g). Water As concentration categories 10 – 20 μg/L and 20 – 50 μg/L were not significantly different from the <10 μg/L category when predicting hair [As], while the >50 μg/L category was highly significant in predicting hair [As] ($p < 0.001$). Figure 4.1 presents a Lowess-smoothed curve of the relationship between water [As] and Hair [As]. There was a high degree of scatter around the line of best fit in Figure 4.1, with few points lying on the line.

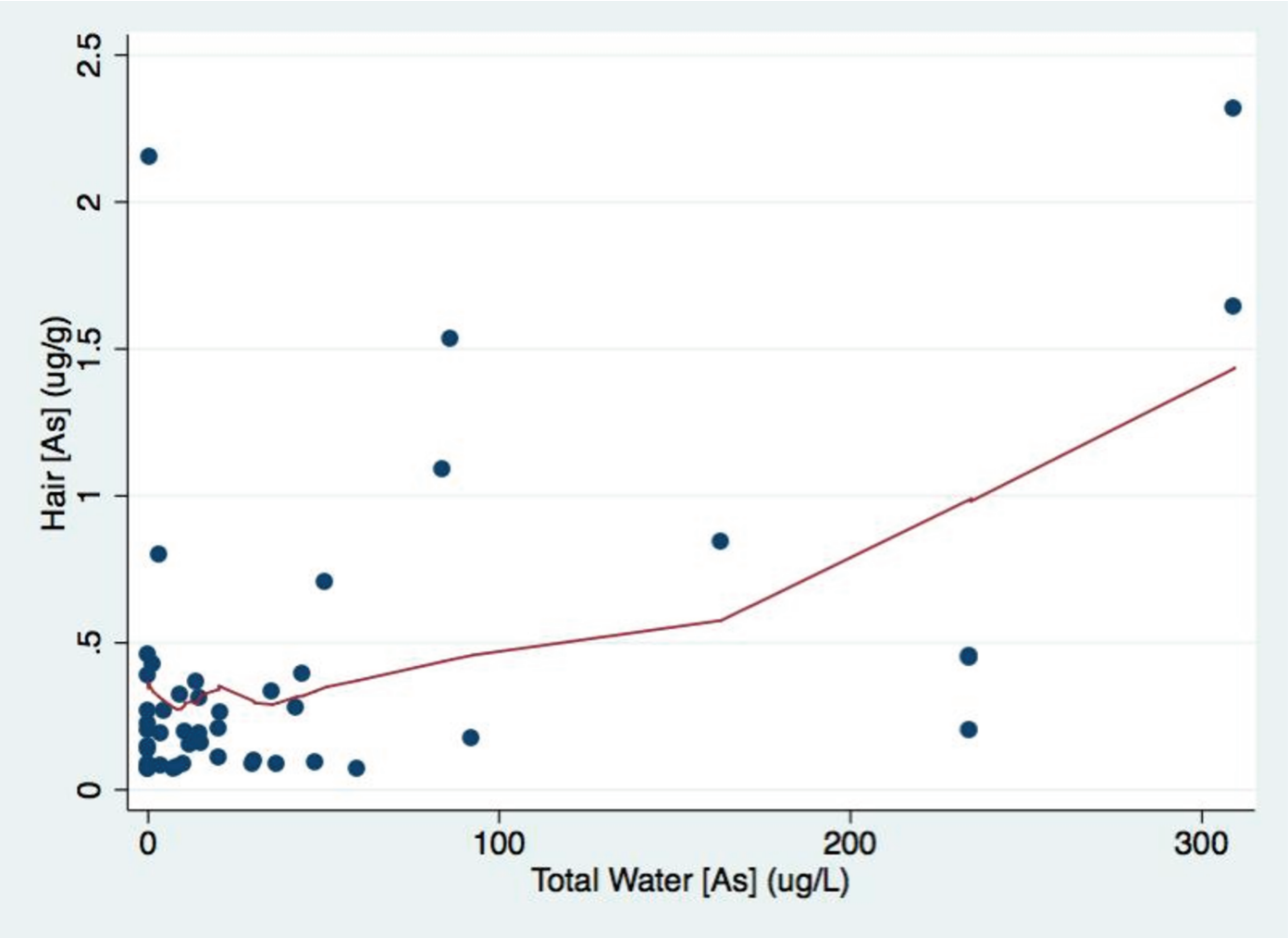

Figure 4.1 Lowess-smoothed curve plot of hair arsenic concentration versus water arsenic concentration, for hair samples with an As concentration of greater than the MDL of 0.068 μg/g (n=48)

4.4.3 Descriptive Statistics of Nail Arsenic Concentrations

A total of 147 and 151 fingernails and toenails were collected, respectfully. Fingernail [As] ranged from less than the MDL of 0.15 μg/g to 4.10 μg/g, and toenail [As] ranged from less than the MDL to 13.18 μg/g. For toenails, this very high [As] of 13.18 μg/g was much larger than the next largest observation, 3.22 μg/g, but was confirmed as being accurate on repeat testing. There were 11 fingernail samples, 9 (82%) from the "exposed" category, which were above the MCL of 1.0 μg/g – 6 from Hubbards and 5 from Fall River. There were 14 toenail samples, 10 (71%) from the "exposed" category, which were above the MCL – 9 from Hubbards and 5 from Fall River. Descriptive statistics of total [As] in nails are presented, for each nail type, in Table 4.6, by location and exposure, for all available samples, and in Table 4.7 by location and exposure, for only samples that had an [As] of greater than the MDL. Shown in Table 4.6 and 4.7, significant differences in both fingernail and toenail [As] were observed between exposure groups, as identified in bold. No significant differences in median [As] were found between locations for any nail group.

Table 4.5 Descriptive statistics of nail arsenic concentrations, by nail type, location, and exposure group

Category	All Nails			Fingernails			Toenails		
	n	Mean (95% CI)	Median (Range)	n	Mean (95% CI)	Median (Range)	n	Mean (95% CI)	Median (Range)
Total	298	0.45 (0.34 – 0.56)	0.22 (0.077* - 13.18)	147	0.40 (0.29 – 0.51)	0.19 (0.077* - 4.10)	151	0.49 (0.30 – 0.68)	0.24 (0.077* - 13.18)
Hubbards	168	0.44 (0.33 – 0.54)	0.22 (0.04 - 4.10)	81	0.46 (0.28 – 0.64)	0.19 (0.077* - 4.10)	87	0.42 (0.29 – 0.54)	0.24 (0.077* - 2.93)
Fall River	130	0.46 (0.25 – 0.67)	0.22 (0.04 - 13.18)	66	0.34 (0.24 – 0.44)	0.20 (0.077* - 2.14)	64	0.59 (0.17 – 1.01)	2.4 (0.077* - 13.18)
Unexposed	176	0.26 (0.20 – 0.33)	**0.077*** (0.077* - 3.60)	89	0.24 (0.077* – 0.33)	**0.11** (0.077* - 3.60)	87	0.29 (0.19 – 0.39)	**0.17** (0.077* - 3.22)
Exposed	122	0.71 (0.47 – 0.96)	**0.37** (0.077* - 13.18)	58	0.66 (0.43 – 0.89)	**0.40** (0.077* - 4.10)	64	0.76 (0.34 – 1.18)	**0.36** (0.077* - 13.18)

Data in μg/g

Bolded data indicate significant differences in medians between categories, within nail type categories ($p < 0.05$)

*Data with an asterisk represent a value of half of the minimum detection limit of As in nails (0.153 μg/g)

95% CI: 95% confidence interval

Table 4.6 Descriptive statistics of nail arsenic concentrations, by nail type, location, and exposure group, for samples with an arsenic concentration of greater than the minimum detection limit (0.153 μg/g)

Category	All Nails			Fingernails			Toenails		
	n	Mean (95% CI)	Median (Range)	n	Mean (95% CI)	Median (Range)	n	Mean (95% CI)	Median (Range)
Total	183	0.68 (0.51 – 0.85)	0.35 (0.16 - 13.18)	82	0.66 (0.48 – 0.84)	0.39 (0.17 - 4.10)	101	0.69 (0.42 – 0.97)	0.34 (0.16 - 13.18)
Hubbards	101	0.67 (0.51 – 0.84)	0.35 (0.17 - 4.10)	44	0.77 (0.46 – 1.08)	0.37 (0.17 - 4.10)	57	0.59 (0.42 – 0.77)	0.34 (0.17 - 2.93)
Fall River	82	0.69 (0.36 – 1.01)	0.34 (0.16 - 13.18)	38	0.53 (0.38 – 0.68)	0.40 (0.17 - 2.14)	44	0.82 (0.21 – 1.43)	0.32 (0.26 - 13.18)
Unexposed	85	0.46 (0.33 – 0.59)	**0.28** (0.17 - 3.60)	39	0.44 (0.26 – 0.63)	**0.28** (0.17 - 3.60)	46	0.48 (0.30 – 0.66)	**0.28** (0.17 - 3.22)
Exposed	98	0.87 (0.57 – 1.16)	**0.44** (0.16 - 13.18)	43	0.86 (0.57 – 1.15)	**0.45** (0.17 - 4.10)	55	0.87 (0.38 – 1.36)	**0.44** (0.16 - 13.18)

Data in μg/g
*Bolded data indicate significant differences in medians between categories, within nail type categories
95% CI: 95% confidence interval

4.4.4 Analytical Statistical Analyses of Factors Associated with Nail Arsenic Concentration

Pearson correlation coefficients between water [As] and nail [As], divided into subcategories based on nail type, water [As], and location, were performed as preliminary analyses. For all nails combined, a Pearson correlation coefficient of 0.1926 was found between water [As] and nail [As]. Fingernails showed substantially higher correlation than toenails (0.3528 vs. 0.1246). Correlations were also higher for Hubbards than Fall River for all nails, fingernails only, and toenails only (0.3326 vs. 0.0856, 0.3680 vs. 0.2178, and 0.3330 vs. 0.0637, respectively). Therefore, separate statistical analyses were done for fingernails and toenails, and location was forced into all linear and logistic models to adjust for location confounding.

Tables 4.7 and 4.8 present results from univariable logistic regression analyses with fingernail [As] and toenail [As] as outcome variables, respectively, dichotomized using the ICP-MS MDL of 0.153 μg/g. In univariable logistic regression analyses, dichotomized fingernail [As] was at least marginally positively associated with seafood consumption, well depth, water pH, hair [As], nail [Se] and [Pb], total As intake and food As intake, and a number of urine and water As metabolite concentrations. Also without considering confounding, using univariable logistic regression analyses, toenail [As] was at least marginally positively associated with age, higher income, water conductivity and pH, hair [As], nail [Se] and [Pb], total As intake, and a number of urine and water As metabolite concentrations.

Table 4.7 Univariable logistic regression analyses for dichotomized fingernail arsenic concentration of greater than the MDL versus less than the MDL of 0.153 μg/g, for variables that were at least moderately significant (p < 0.15)

			Fingernail [As]	
Variable	**n**	**Description**	**OR (95% CI)**	**p-value**
Food As Intake	147	Range: 0 - 2,020.8 μg	1.0011 (1.0002 - 1.0020)	0.020
Hair [As]	128	Range: 0.034* - 2.2 μg/g	7.72 (0.69 – 86.87)	0.098
Nail [Pb]	147	Range: 0.1 - 117.8 μg/g	1.17 (0.97 - 1.42)	0.096
Nail [Se]	147	Range: 0.1 - 28.1 μg/g	1.42 (1.10 – 1.84)	0.007
Seafood consumed (y/n)	147	% Consumed: 37%	2.32 (1.15 – 4.69)	0.019
Seafood consumed (g)	147	Range: 0 – 680.4 g	1.11 (1.02 - 1.20)	0.017
Total As Intake	147	Range: 0 - 3,148.3 μg	1.0010 (1.0002 - 1.0019)	0.019
Urine [As(III)]	126	Range: 0.355 - 19.7 μg/L	1.62 (1.08 – 2.42)	0.020
Urine [DMA(V)]	126	Range: 0.355 - 217.5 μg/L	1.07 (1.01 - 1.12)	0.013
Urine [MMA(V)]	125	Range: 0.355 - 45.3 μg/L	1.25 (0.96 – 1.64)	0.100
Urine [TMA]	125	Range: 0.355 - 293.1 μg/L	1.01 (1.00 - 1.03)	0.102
Urine % As(III)	126	Range: 0 - 22.5%	1.09 (0.97 – 1.22)	0.136
Urine % As(III) – AsB	126	Range: 0 - 24.2%	1.08 (1.00 - 1.17)	0.044
Water [As]	147	Range: 0.008 - 309.2 μg/L	1.02 (1.00 - 1.03)	0.046
Water [As] >10 μg/L (n/y)	147	% >10 μg/L: 40.2%	3.68 (1.79 – 7.56)	< 0.001
Well Depth	133	Range: -130.5 – 62.2 m	1.003 (0.999 - 1.006)	0.117
Water pH	139	Range: 3.2 - 8.4	1.64 (1.04 – 2.58)	0.034

*Data with an asterisk represent a value of one half of the minimum detection limit of As for that sample (MDLs: hair – 0.068 μg/g, urine – 0.709 μg/L, water – 0.017 μg/L)

As(III): arsenite, MMA(V): monomethylarsonic acid, DMA(V): dimethylarsinic acid, TMA: total arsenic minus arsenobetaine, Pb: lead, Se: selenium, 95% CI: 95% confidence interval

Urine % As(III): Percentage of total urinary arsenic accounted for by As(III)

Urine % As(III) – AsB: Percentage of total urinary arsenic, not including arsenobetaine, accounted for by As(III)

Table 4.8 Univariable logistic regression analyses for dichotomized toenail arsenic concentration of greater than the MDL versus less than the MDL of 0.153 μg/g, for variables that were at least moderately significant (p < 0.15)

			Toenail [As]	
Variable	**n**	**Description**	**OR (95% CI)**	**p-value**
Age <50 years	149	% <50 years: 33%	1.89 (0.88 – 4.07)	0.103
Conductivity	142	Range: 28.6 – 1,080 ms	0.998 (0.996 – 1.001)	0.080
Hair [As]	128	Range: 0.034* - 2.2 μg/g	7.72 (0.69 – 86.87)	0.098
Income Category				0.006
<$30,000/year	15		Reference	
$30,000 - $50,000/year	31		10.4 (2.47 – 43.82)	0.001
>$50,000/Year	93		3.64 (1.15 – 11.54)	0.028
Nail [Pb]	151	Range: 0.1 - 117.8 μg/g	3.62 (1.26 – 10.44)	0.017
Nail [Se]	151	Range: 0.1 - 28.1 μg/g	2.11 (1.23 – 3.62)	0.007
Total As Intake	151	Range: 0 - 3,148.3 μg	1.0006 (0.9998 - 1.0015)	0.141
Urine [As]	145	Range: 0.355* – 884.2 μg/L	1.007 (0.999 – 1.014)	0.075
Urine [As(III)]	126	Range: 0.355* - 19.7 μg/L	1.49 (0.98 – 2.78)	0.065
Urine [TMA]	125	Range: 0.355* - 293.1 μg/L	1.03 (1.00 - 1.06)	0.060
Urine % As(III)	126	Range: 0 - 22.5%	1.14 (1.00 – 1.30)	0.048
Water [As]	151	Range: 0.008 - 309.2 μg/L	1.04 (1.01 - 1.07)	0.005
Water [As] >10 μg/L (n/y)	151	% >10 μg/L: 42%	5.45 (2.40 – 12.38)	< 0.001
Water pH	141	Range: 3.2 - 8.4	1.74 (1.06 – 2.85)	0.030

*Data with an asterisk represent a value of one half of the minimum detection limit of As for that sample (MDLs: hair – 0.068 μg/g, urine – 0.709 μg/L, water – 0.017 μg/L)
Pb: lead, Se: selenium, As(III): arsenite, TMA: total arsenic minus arsenobetaine, 95% CI: 95% confidence interval
Conductivity: Water sample electrolytic conductivity (milisiemens)
Urine % As(III): Percentage of total urinary arsenic accounted for by As(III)

Table 4.9 reports multivariable logistic models for predicting the dichotomized [As] in fingernails and toenails.

Table 4.9 Multivariable logistic regression analysis for dichotomized nail arsenic concentration of greater than the MDL versus less than the MDL of 0.153 μg/g

	Fingernails			Toenails		
Variable	**OR (Robust St. Error)**	**95% CI**	**p-value**	**OR (Robust St. Error)**	**95% CI**	**p-value**
Nail [Se] Category			0.003*			0.003*
1	Referent			Referent		
2	3.27 (2.02)	0.98 - 10.98	0.055	1.71 (0.96)	0.57 - 5.14	0.340
3	4.57 (2.78)	1.39 - 15.08	0.013	2.45 (1.24)	0.91 - 6.60	0.076
4	11.63 (7.60)	3.23 - 41.84	<0.001	15.10 (11.30)	3.48 - 65.49	<0.001
Water[As] (>10 μg/L)	4.36 (1.90)	1.85 - 10.25	0.001	6.82 (3.18)	2.74 - 16.99	<0.001
Seafood consumed (oz.)	1.12 (0.05)	1.03 - 1.22	0.008			
Water Se intake (μg)	1.34 (0.39)	0.76 - 2.38	0.316	0.96 (0.03)	0.91 - 1.02	0.217
Location	0.97 (0.42)	0.41 - 2.28	0.938	0.94 (0.41)	0.40 - 2.20	0.879
Constant	0.14 (0.09)	0.02 - 0.46	0.001	0.45 (0.20)	0.19 - 1.08	0.075
n	144			148		
Pseudo-R^2	0.2078			0.2074		

*Overall p-value for categorical variable

Nail [Se]: Nail selenium concentration categories represent quartiles

Fingernail [Se] categories 1 = <0.56 μg/g, 2 = 0.56 – 0.86 μg/g, 3 = 0.87 – 1.89 μg/g, 4 = >1.89 μg/g

Toenail [Se] categories: 1 = <0.57 μg/g, 2 = 0.57 – 0.86 μg/g, 3 = 0.87 – 2.06 μg/g, 4 = >2.06 μg/g

MDL: Minimum detection limit, 95% CI: 95% confidence interval

Location: Hubbards=0, Fall River=1

Table 4.9 Multivariable logistic regression analysis for dichotomized nail arsenic concentration of greater than the MDL versus less than the MDL of 0.153 μg/g (continued)

- Fingernail arsenic logistic model with logit coefficients:
ln[p(fingernail [As] >MDL)/1 – p(fingernail [As] >MDL)] = -2.0 + [1.2*(fingernail [Se] categ. 2)] + [1.5*(fingernail [Se] categ. 3)] + [2.5*(fingernail [Se] categ. 4)] + [1.5*(water [As] >10 μg/L)] + [0.1*(seafood consumed (oz.))] + [0.3*(water Se intake (μg))] + [-0.03*(location)]

- Toenail arsenic logistic model with logit coefficients:
ln[p(toenail [As] >MDL)/1 – p(toenail [As] >MDL)] = -0.8 + [0.5*(toenail [Se] categ. 2)] + [0.9*(toenail [Se] categ. 3)] + [2.7*(toenail [Se] categ. 4)] + [1.9*(water [As] > 10 μg/L)] + [-0.04*(water Se intake (μg))] + [-0.1*(location)]

Multivariable logistic regression analyses for fingernail [As] found that, after controlling for location and Se consumption from water (both non-significant predictors), fingernail [Se], water [As] (as greater or less than 10 μg/L), and the amount of seafood consumed in the previous 3 days were significant predictors. With a fingernail [Se] of < 0.57 μg/g as the baseline, fingernail [Se] in the 0.57 – 0.88 μg/g, 0.89 – 2.25 μg/g, and > 2.25 μg/g categories significantly increased the odds of having a fingernail [As] of greater than the MDL by 3.3 times (95% CI: 1.0 – 11.0), 4.6 times (95% CI: 1.4 – 15.1), and 11.6 times (95% CI: 3.2 – 41.8), respectively, indicating a strong relationship between fingernail [As] and fingernail [Se]. The odds of having a fingernail [As] of greater than the MDL increased by 4.4 times (95% CI: 1.9 – 10.3) if a participant's main source of drinking water contained at least 10 μg/L (p = 0.001). Consumption of seafood (measured in grams consumed in the 3 days prior to questionnaire completion) increased the odds of having a fingernail [As] being greater than the MDL by 1.1 times (95% CI: 1.03 – 1.22) for each additional ounce of seafood consumed (range: 0 – 24 oz.).

The final multivariable logistic regression analyses for toenails showed that after controlling for location and Se consumed from water (both non-significant predictors), toenail [Se] and water [As] were significant positive predictors of the odds of having a toenail [As] of greater than the MDL. With a toenail [Se] of < 0.57 μg/g as the baseline, toenail [Se] in the 0.89 – 2.25 μg/g, and > 2.25 μg/g categories increased the odds of having a fingernail [As] of greater than the MDL by 2.5 times (95% CI: 0.9 – 6.6) and 15.1 times (95% CI: 3.5 – 65.5), respectively, with only the last category being statistically significant (p < 0.001), and the other being

moderately significant ($p = 0.076$). The odds of having a toenail [As] of greater than the MDL was increased by 6.8 times (95% CI: 2.7 – 17.0) if a participant's main source of drinking water contained at least 10 μg/L ($p < 0.001$). In the goodness-of-fit examination of this logistic regression analysis, one observation exhibited very high leverage (0.97, approximately 6.9 times larger than the next highest leverage value). Because there was no identifiable reason for this data point's high leverage, and removing it did not alter the predictor coefficients or pseudo-R^2 significantly, it was left in the final analysis.

Table 4.10 presents univariable linear regression analyses results for natural log transformed fingernail [As] in the sub-population with fingernail [As] of >MDL, for variables of at least moderate significance ($p < 0.15$). Without considering confounding, using univariable analyses, fingernail [As] was at least marginally positively associated with primary school education only, hair [As], nail [Se], total water As intake, and a number of urine and water As metabolite concentrations.

Table 4.10 Univariable linear regression analyses, with natural log transformed fingernail arsenic concentration as an outcome, for samples with more than the MDL, for variables that were at least moderately significant (p < 0.15)

			Fingernail [As]	
Variable	**n**	**Description**	**Coef. (95% CI)**	**p-value**
Education		% Category:		0.066
Primary	7	9%	Reference	
Secondary	23	29%	-0.67 (-1.32 to -0.01)	0.049
College	17	21%	-0.88 (-1.56 to -0.20)	0.012
University	33	41%	-0.81 (-1.44 to -0.17)	0.013
Hair [As]	72	Range: 0.034* – 2.2 μg/g	0.63 (0.09 – 1.16)	0.023
Nail [Se]	82	Range: 0.2 - 28.1 μg/g	0.07 (0.03 - 0.10)	0.001
Urine [As(III)]	73	Range: 0.355* - 19.7 μg/L	0.07 (0.01 - 0.13)	0.019
Urine [As(V)]	73	Range: 0.355* - 20.0 μg/L	0.09 (0.04 - 0.14)	0.001
Urine % As(V)	73	Range: 0 – 74.4%	0.03 (0.01 - 0.05)	0.002
Urine % AsB	73	Range: 0 – 86.5 %	-0.008 (-0.015 to -0.001)	0.028
Water [As]	82	Range: 0.008* – 234.2 μg/L	0.006 (0.003 – 0.010)	0.001
Water [As(V)]	49	Range: 0.5 – 235.7 μg/L	0.004 (-0.001 – 0.009)	0.098
Water As Intake	82	Range: 0 – 1,798.8 μg	0.0014 (0.0007 - 0.0022)	< 0.001

Coef: coefficients - in natural format

*Data with an asterisk represent a value of one half of the minimum detection limit of As for that sample (MDLs: hair – 0.068 μg/g, urine – 0.709 μg/L, water – 0.017 μg/L)

Se: selenium, As(III): arsenite, As(V): arsenate, AsB: arsenobetaine, 95% CI: 95% confidence interval

Urine %: Percentage of total urinary arsenic accounted for by a specific metabolite

MDL: minimum detection limit of 0.153 μg/g

Table 4.11 presents univariable linear regression analyses results for natural log transformed toenail [As] in the sub-population with toenail [As] of >MDL, for variables of at least moderate significance ($p < 0.15$). Without considering confounding, using univariable analyses, toenail [As] was at least marginally positively associated with water softener use, well depth, nail [Se] and [Pb], total As intake, and a number of urine and water As metabolite concentrations.

Table 4.11 Univariable linear regression analyses, with natural log transformed toenail arsenic concentration as an outcome, for samples with more than the MDL, for variables that were at least moderately significant (p < 0.15)

			Toenail [As]	
Variable	**n**	**Description**	**Coef. (95% CI)**	**p-value**
Nail [Pb]	101	Range: 0.1 – 36.7 μg/g	0.09 (0.07 - 0.12)	< 0.001
Nail [Se]	101	Range: 0.1 - 27.3 μg/g	0.10 (0.07 - 0.13)	< 0.001
Water Softener	99	No – 79, Yes – 20	0.32 (-0.07 - 0.71)	0.110
Total As Intake	101	Range: 0 - 3,148.3 μg	0.0003 (0.0001 - 0.0005)	0.025
Urine [As(III)]	84	Range: 0.355* - 19.7 μg/L	0.10 (0.04 - 0.16)	0.001
Urine [As(V)]	83	Range: 0.355* - 20.0 μg/L	0.09 (0.03 - 0.14)	0.001
Urine [DMA(V)]	84	Range: 0.355* - 217.5 μg/L	0.010 (0.003 - 0.016)	0.003
Urine [MMA(V)]	84	Range: 0.355* - 45.3 μg/L	0.05 (0.02 - 0.08)	0.005
Urine [TMA*]	84	Range: 4.2 - 293.1 μg/L	0.005 (0.001 - 0.009)	0.030
Urine % As(III)	84	Range: 0 – 22.5 μg/L	0.04 (-0.01 – 0.09)	0.090
Urine % As(V)	84	Range: 0 – 74.4 μg/L	0.03 (0.01 – 0.06)	0.004
Urine % DMA(V)	84	Range: 0 – 79.7 μg/L	0.008 (-0.001 – 0.016)	0.065
Urine % MMA(V)	84	Range: 0 – 18.4 μg/L	0.04 (0.01 – 0.08)	0.026
Urine % AsB	84	Range: 0 – 86.5 μg/L	-0.0067 (-0.0133 to -0.0001)	0.049
Water [As]	101	Range: 0.008* – 309.2 μg/L	0.002 (-0.001 – 0.005)	0.104
Water [As(V)]	64	Range: 0.1 – 249.0 μg/L	0.005 (0.001 – 0.009)	0.022
Well Depth**	93	Range: -130.5 – 62.2 m	0.0013 (0.0001 – 0.0026)	0.034

Coef: Coefficients in natural log format

*Data with an asterisk represent a value of half of the detection limit of As for that sample (MDLs: urine – 0.709 μg/L, water – 0.017 μg/L, nails – 0.153 μg/g)

Pb: lead, Se: selenium, As(III): arsenite. As(V): arsenate, DMA(V): dimethylarsinic acid, MMA(V): monomethylarsonic acid, TMA: total minus arsenobetaine, 95% CI: 95% confidence interval

Water Softener: A water softening device was used on the main source of drinking water, 0 = no, 1 = yes

Urine %: Percentage of total urinary arsenic accounted for by a specific metabolite

*Well depth expressed relative to sea level. Topographical information (household elevation) was combined with well depth (below ground level) to obtain the position of the well relative to sea level.

Table 4.12 reports multivariable linear regression models for predicting natural log transformed [As] in fingernails and toenails for the sub-population with nail [As] of >MDL. Coefficients and confidence intervals for the linear regression analysis are in natural log transformed format to account for non-normally distributed nail [As]. All linear regression models met assumptions of independence, linearity and homoscedasticity (p-values >0.15).

Table 4.12 Multivariable linear regression analyses, with natural log-transformed nail arsenic concentration as an outcome, for nail samples with an arsenic concentration greater than the MDL (0.153 μg/g)

	Fingernails			Toenails		
Variable	**Coef. (Robust St. Error)**	**95% CI**	**p-value**	**Coef. (Robust St. Error)**	**95% CI**	**p-value**
Nail [Se] Category			<0.001*			<0.001*
1	Referent			Referent		
2	0.29 (0.17)	-0.05 - 0.62	0.089	0.31 (0.24)	-0.16 - 0.78	0.189
3	0.18 (0.15)	-0.12 - 0.47	0.237	0.30 (0.16)	-0.02 - 0.61	0.063
4	1.01 (0.20)	0.60 - 1.41	<0.001	0.98 (0.22)	0.55 - 1.41	<0.001
Water [As] Category			<0.001*			0.029*
1 - <10 μg /L	Referent			Referent		
2 - 10 - 20 μg/L	0.22 (0.19)	-0.17 - 0.60	0.265	0.31 (0.19)	-0.06 - 0.69	0.103
3 - 20 - 50 μg/L	0.57 (0.15)	0.28 - 0.86	<0.001	0.36 (0.19)	-0.01 - 0.74	0.056
4 - >50 μg/L	0.99 (0.21)	0.58 - 1.41	<0.001	0.76 (0.28)	0.21 - 1.31	0.008
Location	-0.06 (0.13)	-0.33 - 0.20	0.642	-0.08 (0.15)	-0.38 - 0.21	0.581
Water Se Intake Category						0.242*
1	-	-	-	Referent		
2	-	-	-	0.08 (0.20)	-0.31 - 0.48	0.682
3	-	-	-	-0.18 (0.18)	-0.54 - 0.18	0.317
4	-	-	-	0.15 (0.23)	-0.32 - 0.61	0.525
Water Se intake (μg)	-0.003 (0.004)	-0.010 - 0.004	0.406	-	-	-
Constant	-1.44 (0.10)	-1.63 to -1.24	<0.001	-1.51 (0.22)	-1.94 to -1.07	<0.001
n	81			99		
Adjusted-R^2	0.4691			0.2927		

*Overall p-value for categorical variable
Coefficients in natural log format, 95% CI: 95% confidence interval

Table 4.12 Multivariable linear regression analyses, with natural log-transformed nail arsenic concentration as an outcome, for nail samples with an arsenic concentration greater than the MDL (0.153 μg/g) (continued)

Nail [Se]: Nail selenium concentration

- Fingernail [Se] categories – 1 : <0.70 μg/g, 2: 0.70 – 1.20 μg/g, 3: 1.21 – 2.60 μg/g, 4: >2.60 μg/g; range: 0.1 – 28.1 μg/g
- Toenail [Se] categories – 1: <0.64 μg/g, 2: 0.64 – 0.85 μg/g, 3: 0.86 – 2.11 μg/g, 4: >2.11 μg/g; range: 0.1 – 27.3 μg/g

Location: Hubbards=0, Fall River=1

Water Se intake categories:

- Toenail model: 1 = <0.04 μg, 2 = 0.04 – 0.10 μg, 3 = 0.11 – 0.26 μg, 4 = >0.26 μg

- Fingernail arsenic concentration model with back-transformed coefficients:

  Fingernail [As] = 0.2 * [1.3*(nail [Se] categ. 2)] * [1.2*(nail [Se] categ. 3)] * [2.75*(nail [Se] categ. 4)] * [1.3*(water [As] categ. 2)] * [1.8*(water [As] categ. 3)] * [2.7*(water [As] categ. 4)] * [0.9*(location)] * [1.0*(water Se intake (μg))] + error

- Toenail arsenic concentration model with back-transformed coefficients:

  Toenail [As] = 0.2* [1.4*(nail [Se] categ. 2)] * [1.4*(nail [Se] categ. 3)] * [2.7*(nail [Se] categ. 4] * [1.4*(water [As] categ. 2)] * [1.4*(water [As] categ, 3)] * [2.1*(water [As] categ. 4)] * [0.9*(location)] * [1.1*(water Se intake categ. 2)] * [0.8*(water Se intake categ. 3)] * [1.2*(water Se intake categ. 4)] + error

The final multivariable linear regression model for fingernail [As] for samples with an [As] of >MDL showed that, after controlling for location and Se consumption from water (both non-significant predictors), fingernail [Se] (categorized into 4 equal groups), and water [As] (as a categorical variable) were significant predictors of fingernail [As]. Participants whose fingernails contained greater than 2.60 μg/g of Se were predicted to have a significantly higher [As] in their fingernails, by 2.8 times (95% CI: 1.8 – 4.1), compared to participants with less than 0.70 μg/g of Se in their fingernails. As well, participants whose home tap water contained between 20-50 μg/L or >50 μg/L of As were predicted to have fingernail [As] that were 1.8 times (95% CI: 1.3 – 2.4) and 2.7 times (95% CI: 1.8 – 4.1) higher than those with water [As] of less than 10 μg/L. Lowess-smoothed plots depicting the relationship between fingernail [As] and fingernail [Se] and water [As] are presented in Figure 4.2.

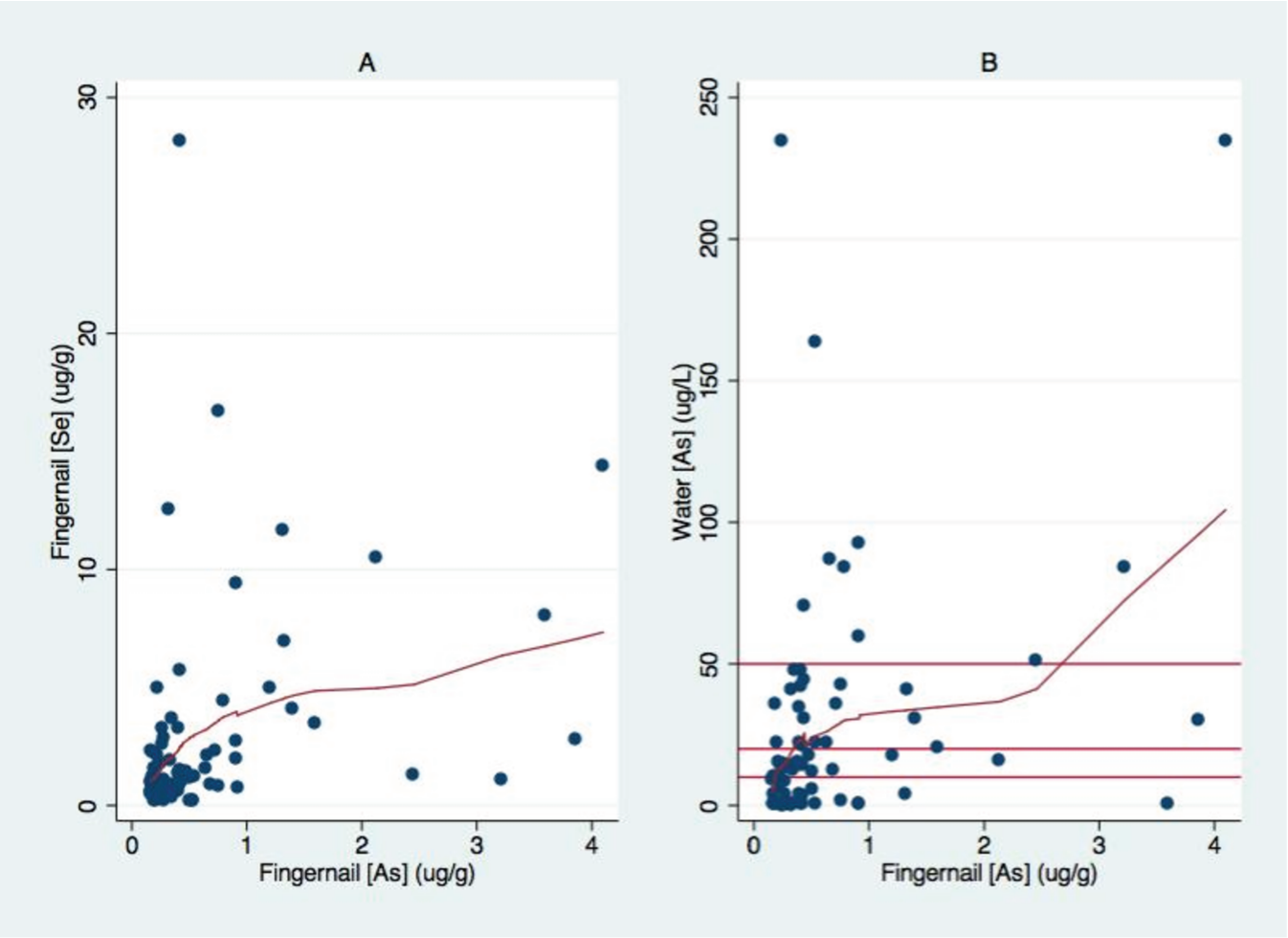

Figure 4.2 Lowess-smoothed curve plots of the relationship between A) fingernail [As] and fingernail [Se] (n=81), and B) fingernail [As] and water [As] (n=81). Reference lines placed at water [As] of 10, 20, and 50 μg/L.

At higher nail [As], there was considerable error around the line of best fit for both fingernail [Se] and water [As]. Distinctly different slopes of the line of best-fit can been seen in different water [As] sections of the graph, <20 μg/L, 20 – 50 μg/L, and >50 μg/L, as indicated by the horizontal lines in Figure 4.2-B.

The final multivariable linear regression analysis for toenail [As] showed that, while again controlling for location and Se consumption from water (non-significant predictors), toenail [Se] and water [As] were significant predictors. Participants whose toenails contained greater than 2.11 μg/g of Se were predicted to have a significantly higher toenail [As], by 2.7 times (95% CI: 1.7 – 4.1), than those with less than 0.64 μg/g of Se in their toenails. Furthermore, participants whose drinking water contained more than 50 μg/L were predicted to have significantly higher concentrations of As in their toenails, by 2.1 times (95% CI: 1.2 – 3.7), than those with less than 10μg/L of As in their water. In this analysis, the 10 – 20 μg/L and 20 – 50 μg/L water [As] categories were borderline positively significant (p = 0.103 and p = 0.056, respectively). Figure 4.3 presents Lowess-smoothed curves of the relationships between toenail [As] and toenail [Se] and water [As].

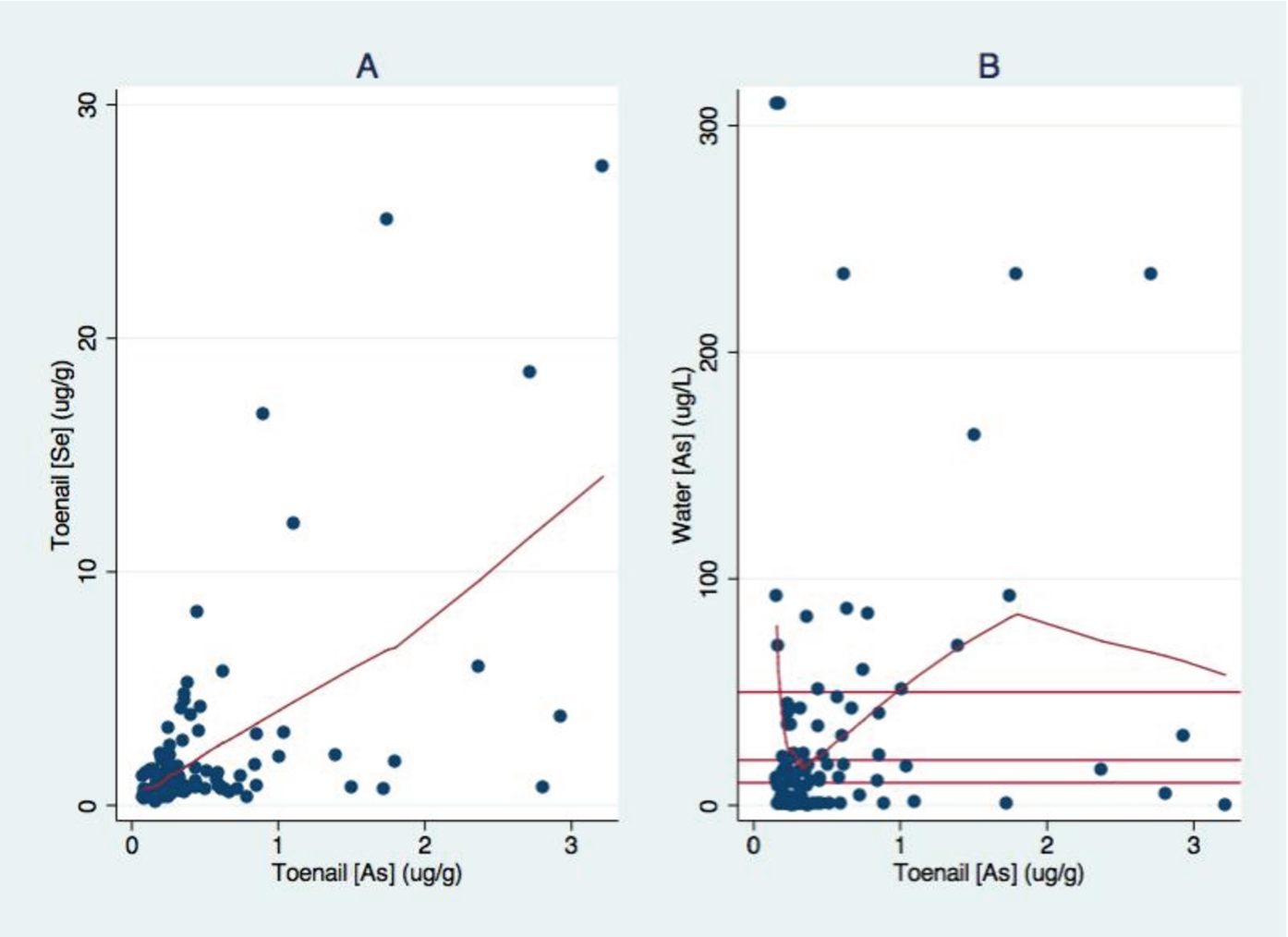

Figure 4.3 Lowess-smoothed curve plots of the relationship between A) toenail [As] and toenail [Se] (n=101), and B) toenail [As] and water [As] (n=101), with one large outlying toenail observation ([As] = 13.18 μg/g) removed for clarity. Reference lines placed at water [As] of 10, 20, and 50 μg/L.

In Figure 4.3-A, toenail [Se] showed a nearly linear increase with toenail [As], with the [As] increasing by approximately 2.5 μg/g for every 10 μg/g increase in [Se] (Se range was 0.1 – 27.3 μg/g), although there was substantial variability around the line of best-fit, especially at the upper concentrations for both elements. Water [As] showed a roughly linear, positive relationship to toenail [As] between approximately 0.3 and 1.9 μg/g, encompassing the 20 – 50 and the >50 μg/L water [As] categories. At higher toenail [As], the curve appears to level off, or even decrease, but there is much variability around the line of best fit, and there are very few data points in this high range.

4.5 Discussion

For both hair and nail samples, the concentration of Se in each sample was found to have a highly significant positive association with the concentration of As in that sample. Water [As] was also significantly positively associated with the concentration of As found in hair and nail samples. Surprisingly, logistic regression analyses suggested that seafood consumption was positively associated with the [As] found in fingernails.

Hair [As] from this study ranged from less than the detection limit of 0.068 μg/g to 2.32 μg/g (Table 4.1). These values were representative of the ranges reported in other studies of hair [As] from both exposed and non-exposed populations (Gault et al., 2008; Mandal et al., 2003; Mosaferi et al., 2005). Similarly, fingernail [As] ranged from less than the detection limit of 0.153 μg/g to 4.10 μg/g, and toenail [As] ranged from <MDL to 13.18 μg/g. The [As] in fingernails and

toenails in this study were consistent with the ranges reported by other researchers in both exposed and non-exposed populations (Gault et al., 2008; Karagas et al., 1996, 2000; Mandal et al., 2003; Wilhelm et al., 2005).

A person's methylation capacity (the ability to methylate inorganic As to organic, methylated forms of As) may be an influencing factor in the [As] found in hair, because most of the As in hair is in the inorganic form (Mandal et al., 2004); therefore, a person with low methylation capacity may have more circulating iAs available for incorporation into hair. Overall, the Pearson correlation coefficient between hair [As] and water [As] was 0.576 . This is much lower than correlation values reported by Gault et al. who found a correlation of 0.86 in a Cambodian study (Gualt et al., 2008), or by Wang et al. who found a Pearson correlation of 0.78 ($p < 0.05$) in a study of arsenicosis in China (Wang, Yang, Hou, et al., 2001). The finding that Pearson correlation coefficients between water [As] and hair [As] were higher in Hubbards than in Fall River (Section 4.4.2) may suggest that there were more unmeasured sources of As exposure in the Fall River population than in the Hubbards population. Estimated As consumption from food sources was not a significant predictor in our multivariable analyses, although measurement error associated with food consumption estimation could have led to a type II error. Similarly, there has been documented evidence of As in the soil in areas near Fall River, as a result of historic gold-mining operations, but not in Hubbards (Meunier, Kock & Reimer 2009; Meunier, et al. 2010). Previous research has shown that As from external sources can be absorbed by hair, after which washing techniques are able to remove only a small amount of externally absorbed As, thereby leading to

possibly inaccurate measurements of internal exposure (Hindmarsh, 2002; Smith & Hendry, 1934). Directly measuring actual As consumption from foods, soil and air [As] were not objectives or budget items of this project, which may limit the interpretation of the results.

In our study, there was a Pearson correlation coefficient of 0.353 between water [As] and fingernail [As] (Section 4.4.4), with a higher correlation found between the two in Hubbards than in Fall River (0.368 vs. 0.218). In previous studies, the correlation between water [As] and fingernail [As] ranged from 0.74 (Gault et al., 2008), while Mandal et al. reported a correlation of 0.68 (Mandal et al., 2003). The Pearson correlation coefficient between water [As] and toenail [As] was 0.125, with Hubbards again showing a higher correlation between the two than Fall River (0.333 vs. 0.064). Correlations between water [As] and toenail [As] have been reported to range from 0.55 to 0.67 (Hinwood et al., 2003; Karagas et al., 1996; Karagas et al., 2000). The discrepancy found in this study between correlations between locations may be attributed to external exposure, as was suggested above for hair samples, although if this were the case, one would expect the fingernail:toenail Pearson correlation coefficient ratio to be reversed. It has been reported that toenails, in comparison to fingernails, are superior biological markers of As exposure because they are less prone to external contamination from water, air, soil and dust (Garland et al., 1993; Morris, Stampfer, & Willett, 1983). Following this rationale, it would be expected that toenail [As] would have a higher correlation with water [As] than would be found for fingernails; however, we found that for As consumed from water or food, fingernail [As] was consistently

more associated with both than toenail [As] (see chapter 2). The reason for this finding is unknown, though it may be due to the relationship between the short-term consumption information and the relatively long-term toenail As deposition (longer than fingernails due to the slower growth).

Since water exposure to As was the focus of our project, we examined other routes of water exposure to As. It has been shown that hair can absorb As by washing in As contaminated water (Harrington, Hiddaugh, Morse, et al., 1978). In initial multivariable analyses, however, frequency of bathing/hair washing was a non-significant predictor of hair [As], possibly due to little variation in this variable between locations, and was not included in future analyses. Information about household outdoor gardening was also collected during the interview questionnaire, but no gardening-related variables were significant in any regression analyses to predict hair [As].

In the prediction of both hair (Tables 4.3 and 4.5) and nail [As] (Tables 4.9 and 4.12), linear and logistic models found water [As] to be a significant predictor. Many studies have found significant positive relationships between both hair and nail [As] and the [As] in a person's primary drinking water source (Cavar, Klapec, Grubesić, et al., 2005; Hinwood et al., 2003; Mandal et al., 2003; Shraim et al., 2003). Arsenic consumed from water is dissolved in the blood, which nourishes the hair follicle bulb and nail bed, and therefore As is deposited in hair and nail as they grow (Pearson & Pounds, 1971; Stenn, 1991). Additionally, As has a strong affinity for tissues that are rich in keratin, including hair, nails, and skin, making these

tissues of special interest for biomonitoring (Hindmarsh, 2002; Marchiset-Ferlay et al., 2012; Orloff et al., 2009; Slotnick & Nriagu, 2006).

Our research has found that, besides water [As], the [Se] in hair, fingernails, or toenails was a major positive predictor of the [As] in each respective biological sample, even while taking Se consumed from water into account (Tables 4.3, 4.5, 4.9, and 4.12). Because it has been previously demonstrated that an increase in Se in the diet can help protect against the toxic outcomes of As exposure (Wang et al., 2001), we believe this is a significant finding, and that future research using hair and nails as biomarkers of As exposure should pay special attention to factors related to Se consumption and its concentration in biological tissues relative to As. Ingestion or injection of one element often results in the increased excretion of the other (Biswas, Talukder, & Sharma, 1999; Gailer, 2007; Wang et al., 2001). Of particular interest is the arsenic-selenium conjugate compound, seleno-bis (S-glutathionyl) arsinium ion $[(GS)_2AsSe]^-$, which has been reported to likely form in the liver and blood of mammals, and is suggested to be a primary source of the mutual antagonistic excretion pattern between the two elements (Gailer et al., 2002). Formation of $(GS)_2AsSe^-$ in the liver and blood decreases the amount of Se excreted through exhalation (a major pathway of Se excretion under normal physiological conditions), and increases its biliary excretion and subsequent release through the gastrointestinal tract, after injection with sodium arsenite (Gailer et al., 2000, 2002; Gailer, 2007, 2009). Although it has not been reported previously, we hypothesize that it is possible that $(GS)_2AsSe^-$ is being incorporated into hair and nails as a mechanism of mutual detoxification. While $(GS)_2AsSe^-$ is an

organic compound, and inorganic As species – As(III) and As(V) – are the dominant form of As in hair and nail samples, organic As forms such as MMA(V), DMA(III), and DMA(V), have also been measured in hair and nails, albeit at lower concentrations than inorganic As species (Mandal et al., 2003). Further research into their interactions in hair and nails is warranted. Specifically, it would be beneficial to perform more studies which include the speciation of both As and Se in hair and nail tissues, and to discover if the $(GS)_2AsSe^-$ conjugate compound is in fact responsible for detoxification via excretion of As and Se in hair and nails.

There was a roughly linear, positive relationship between the amount of seafood consumed in the 3 days prior to the interview and the odds of having a detectable [As] in fingernails (Table 4.9). This was an unexpected result, as seafood consumption has not typically been considered a viable predictor of nail [As]. Seafood can contain many different types of As, but it is largely believed that As derived from seafood is non-toxic in nature (Andrewes et al., 2004; Sabbioni et al., 1991; Schoof & Yager, 2007). Some types of As derived from seafood have been shown to be positively correlated with, and possibly metabolized to, DMA(V) (Choi et al., 2010; Navas-Acien, Francesconi, Silbergeld, et al., 2011), which could account for the relationship between seafood consumption and fingernail [As]. Normally, however, nails have only a very small percentage of total As in the form of DMA, typically around 3% (Mandal et al., 2003), which is unlikely to account for the strength of the relationship found in this study. There was a significant relationship between the amount of seafood consumed and the urinary [As(III)] (see chapter 3). This relationship may be indicative of seafood- derived As being metabolized into

inorganic As(III), and perhaps this explains the relationship found here between seafood and fingernails [As], since inorganic As, and especially As(III) , is a major component of the total [As] in nails (Mandal et al., 2003). Elucidation of the pathway used to metabolize seafood derived As may help to shed light on these findings.

As discussed in Section 2.5, linear and logistic regression models including random effects variables, to account for potential clustering at the household and location levels, were explored but they did not significantly contribute any information that was not found in models without random effect variables. Therefore, the simpler models with only fixed effect variables were discussed. Fixed effects models were fitted using robust standard errors, to incorporate some of the minimal effect that clustering may have had on the dataset.

There are several potential sources of bias that should be considered when interpreting the results of this study. The questionnaire used for this study to estimate the amount of As each person ingested from different sources was only a snapshot in time of a person's normal eating and drinking habits. Information on water consumption was collected for the previous two days, and for the previous three days for food consumption, with longer durations being susceptible to recall bias. Over such a short time period, it is difficult to assess the average or "normal" As ingestion rate over a longer period of time. For this reason, attempting to correlate short-term ingestion with longer-term biomarkers of exposure such as fingernails, toenails, and hair may be less than ideal. To more accurately assess the relationship between long-term biomarkers and As ingestion, it may be necessary

to consider As ingestion over a longer time period. In addition, although standard procedures for hair collection have been proposed, many studies do not follow suggested guidelines, as is evident from different sample collection strategies in published literature (Gault et al., 2008; Hindmarsh, 2002; Mosaferi et al., 2005; Muikku et al., 2009). In this study, collected samples were not uniform in length, therefore samples represented different periods of exposure, and this probably contributed to some random error in predicting hair [As] in regression analyses.

The questionnaire obtained information on estimated amounts of foods eaten that may contribute to total As consumed, such as foods boiled in water, hot cereals with water added, etc. These additional foods, or aggregates of them, were not significantly associated with any biological samples' [As] in any analyses. It is difficult to assess the level of As exposure with these types of foods because of variations between people on how much water was used to prepare each type of food, water to weight ratio at consumption, and other factors.

Due to time constraints during field data collection, many participants had biological samples collected less than 48-hours after they were asked to participate in the study, and because of this, some nail samples were of insufficient mass to be analyzed for the presence of As. As well, participants with little advanced warning of sample collection were often unable to reliably recall what they had eaten or drank in the last 3 days, whereas participants with ample warning were able to make mental or physical notes on what they had eaten, perhaps biasing the results. Additionally, we wanted a large number of participants with water samples containing greater than 10 μg/L of As, and so every household in the screening

stage in this category was contacted. In contrast, only a fraction of participants in the <10 μg/L of As category were contacted, albeit by random selection. This technique of participant selection may have introduced some selection bias into the study. As well, when individuals were contacted to request that they take part in the study, they were informed of their water [As]. For participants whose water was above the MCL of 10 μg/L, an adjustment of normal drinking habits may have occurred in the time between recruitment and sample collection. This could be especially important when comparing long-term biomarkers, such as hair and nails, to the amount of As consumed from water in the last 2 days, which was taken to be representative of "normal" exposure for each individual.

4.6 Conclusions

The same two predictive factors were found to be positively associated with the [As] found in hair, fingernails, and toenails: [As] in a person's main source of drinking water, and [Se] in each respective biological sample. Additionally, the consumption of seafood was found to be positively predictive of high [As] in fingernails, suggesting that As derived from seafood may have been metabolized into a more bioavailable metabolite, such as toxic As(III). Further research is warranted into the role Se plays in As toxicity and mediation, its role in As sequestration in hair and nail, and the metabolism of seafood derived arsenic.

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5 Evaluation of a Novel X-ray Fluorescence Diagnostic Test for Arsenic Exposure in Human Nail Samples

5.1 Abstract

A method of arsenic (As) detection in fingernails and toenails by use of x-ray fluorescence (XRF) was evaluated in this study. Nail samples were measured for As and selenium (Se) concentration by x-ray fluorescence (XRF), and water and nails were tested by inductively coupled plasma-mass spectrometry (ICP-MS). Selenium was measured due to interactions between As and Se metabolism, including antagonistic effects between these elements in urine and tissues.

Using concordance correlation coefficient (CCC) analyses, kappa analyses, sensitivity (Sn) and specificity (Sp) analyses, and linear regression analyses, the nail [As] and [Se] measured by XRF were compared to those of ICP-MS. It was found that an increased element concentration (As or Se) was related to increased agreement between test methods, as was increased sample mass. The CCC peaked for scaled values of fingernail samples, at 0.560 (95% CI: 0.172 – 0.948). The largest kappa value, 0.636 (95% CI: -0.006 – 0.1000), was found at a 2.0 μg/g cut-off concentration, for fingernails only, and the largest kappa at a clinically relevant cut-off concentration of 1.0 μg/g was 0.237 (95% CI: -0.068 – 0.543), again in fingernails (neither was statistically significant). Analyses for both As and Se showed high XRF Sn, of up to 100% (95% CI: 48 – 100%) but low Sp. Regression analyses found that increasing sample mass was associated with a decrease in the

difference between nail As and Se concentration measurements by ICP-MS and XRF.

While this novel, non-invasive method of As and Se detection has shown relatively high agreement in a select few scenarios, it was determined that the XRF is not currently suitable as a replacement for ICP-MS.

5.2 Introduction

There have been many epidemiological investigations into the health effects of As, resulting in clear evidence that chronic exposure to high concentrations of As can lead to a greater risk of different types of cancers, including: skin, kidney, urinary bladder, and lung. (Banerjee et al., 2008; Chiou et al., 2001; Ferreccio et al., 2000; Guo, Chiang, Hu, et al., 1997; Kurttio, Pukkala, Kahelin, et al., 1999; Yu, Liao & Chai, 2006). Recently the World Health Organization reduced the maximum contaminant level (MCL) of As in drinking water by eighty percent, from 50 μg/L to 10 μg/L (World Health Organization (WHO), 2008). While water sources of As are environmental concerns, As can also be ingested through food sources or via inhalation. Foods and drinks such as chicken, rice, apple juice and seafood can sometimes have [As] of concern (Dabeka et al., 1993; Schoof et al., 1999). Inhalation of As in a rural or residential environment is typically low but can reach dangerous levels near some areas of industrial activity, particularly gold mining (European Commission, 2001). However, many areas of the world have neither the knowledge nor the resources to assess the concentrations of As in their drinking water, food or air, or those found in human populations. Areas of particular

concern are low socio-economic regions of India, Bangladesh, Mongolia, and Taiwan, and certain areas of China and South America, as well as others.

It has been known for some time that there are interactions between As and Se in biological systems, and each element has a history of being used as a treatment for toxicity caused by the other (Biswas, Talukder & Sharma, 1999; Levander, 1977). Additionally, it has been shown that As and Se act as antagonists in higher mammals and that an increased As intake can decrease Se uptake, and vice versa (Gailer, 2007; Gailer et al., 2000). In a controlled trial in Mongolia, Yang et. al. found that, after switching primary drinking sources from As-contaminated drinking water (>50 μg/L) to non-contaminated water (< 50 μg/L, the Mongolian MCL for As in water), people that were supplied with 100-200 μg/day of Se-enriched yeasts had lower concentrations of As in blood and hair compared to a control group. The Se-supplemented group had As decreases in hair from 2.57 μg/g to 0.68 μg/g, while the control group had decreases of As in hair from 2.62 μg/g to 1.25 μg/g (Yang, Wang, Hou, et al., 2002). Due to this interaction between As and Se, both elements were of interest in the evaluation of XRF in comparison to ICP-MS.

Epidemiological researchers and public health practitioners require an accurate, precise, practical, and cost-effective method for estimating As exposure. Assessing the degree to which humans have been exposed to As can be achieved in several ways. The most obvious method is to analyze a person's drinking water for the presence of As, which can be accomplished by use of an inductively coupled

plasma mass spectrometer (ICP-MS) (United States Environmental Protection Agency, 1994). After determining how much As is present in a person's water supply, investigation of the amount of this water (and other liquids mixed with this water, or foods cooked or processed with this water) that is consumed will estimate an average daily consumption of As from this water. Although this method can estimate As exposure from a personal water supply, it is not able to capture any exposure to As that may occur outside of the home, unless other water sources outside the home are tested as well. This method also does not account for As exposures from food, or dermal or inhaled exposure.

Another method of estimating As exposure is to determine the concentration of As in various biomarkers. Once ingested and metabolized by the body, most As is rapidly excreted in the urine. It was found that after a single 500 μg dose of sodium arsenite, approximately half of the As excreted in urine over a 4-day period was excreted in the first 28 hours. After this 28-hour period, urinary [As] steadily tapered off (Buchet, Lauwerys & Sharma, 1981). Due to its ability to create strong bonds with sulfhydryl groups, As binds preferentially with keratin, and therefore As is also excreted in small amounts in hair and nails (Hopps, 1977). Using ICP-MS, the concentration of total As in hair, nails and urine samples can be determined.

The use of x-ray fluorescence (XRF) to detect As and Se in human biological samples is still in its infancy, but it may be a useful alternative to ICP-MS. The concept of using XRF to detect As is based on the fact that when an atom (e.g. As) absorbs a photon, an electron is ejected from the inner shell, which leads to the

emission of an x-ray when a higher orbital electron fills the vacancy. Each x-ray energy is specific for each element (a so-called "characteristic x-ray"), and thus XRF is able to distinguish one element from another (Studinski, McNeill, Chettle et al., 2005).

Presently, the only published data on the use of XRF to detect As or Se in human biological samples are from several papers using "phantoms" as surrogates to human tissue (Fleming & Gherase, 2007; Roy, Gherase, & Fleming, 2010; Studinski et al., 2005). Phantoms are created using polyester resins that are made to mimic human skin. A known amount of As, or any element, is added to the phantom prior to exposure to an appropriate x-ray beam. After exposing the phantom to the x-ray beam, the known concentration of the phantom is compared to the detected amount inferred from XRF. An XRF procedure was reported to be capable of detecting As at concentrations of 0.446 ± 0.006 μg/g in skin phantoms after 120 seconds of exposure time (Fleming et al., 2007). However, there are no reports of validation of this test method in human biological samples.

The use of an XRF technique to measure [As] in human biological samples presents several advantages over traditional methods of measurement. An XRF method, used in a field setting, requires no sample preparation and only approximately 180 seconds for a sample to be run, followed by approximately 15 minutes of data analysis. Conversely, it takes approximately 5 minutes to run a similar sample through ICP-MS, but this does not take into account the time required to ship samples from the collection location to a laboratory or preparation

of the sample prior to analysis (up to 2 hours for sample preparation, as described below). As well, to quantify the amount of both As and Se in a sample by XRF, the cost of analysis is approximately $10, whereas the same cost is associated with the quantification of a single element by ICP-MS analysis, and increases to approximately $15 for multi-element analysis. Furthermore, the initial purchase cost of the XRF is approximately $50,000, compared to approximately $250,000 for ICP-MS. Finally, the XRF device is battery operated and readily deployable in the field, so remote areas that are far from a traditional and well-stocked laboratory would become more available to testing. In contrast, an ICP-MS requires a constant energy source and sterile laboratory environment to function properly.

The objective of this study was to compare the fingernail and toenail results from As and Se testing using ICP-MS to a novel method of detection, XRF, to determine the XRF functional range and operating characteristics, for the As and Se exposure levels found in the province of Nova Scotia, Canada. Presently, the XRF system used in this study is not capable of measuring As in liquid samples, as fluids scatter the x-rays and materials used to contain liquid samples decrease the returned signal intensity, making detection difficult. Hair samples were not feasible to be analyzed by XRF due to the very low mass of samples. The accuracy of XRF depends highly on a suitable mass for each sample and hair samples did not meet this requirement.

5.2 Materials and Methods

5.2.1 Screening Procedure to Identify a Sampling Frame and Study Groups

See section 2.3.1 of this thesis for methods pertaining to the identification and selection of sampling frame and study groups, respectively.

5.2.2 Laboratory Analysis

In July of 2010, fingernail, toenail, hair, urine samples and a second water sample were collected from study participants during a face-to-face interview. Urine and hair samples were collected as part of a larger study, and were not used for the comparison of ICP-MS and XRF. Nail samples were collected by a trained public health nurse using stainless steel nail clippers, which were disinfected with sterile alcohol wipes between each participant. Samples were collected into Ziploc bags and remained on ice or refrigerated until processing.

After collection and pre-processing, the fingernail and toenail samples were sent to Acadia University, in Wolfville, NS, to be washed (details below) before being sent to Mount Allison University, in Sackville, New Brunswick, for analysis of total As and Se by use of a novel X-ray fluorescence (XRF) technique. The operating procedure for the XRF analysis is described in detail elsewhere (Fleming et al., 2007), but will be described here in brief (typical energy spectra and calibration curves for XRF are presented in Appendix 3). An Innov-X Alpha-4000S model analyzer (Innov-X Systems Canada, Mississauga, Ontario) was used to perform the x-ray fluorescence analysis on the collected nail samples. The source of x-rays was a miniature x-ray tube operating at a voltage of 40 kV and a current of 20 μA. Nail

samples were placed in the center of the x-ray window and data on the concentration of As was collected. Each analysis took 180 seconds to complete, and each nail sample was analyzed 5 times, for a total of 15 minutes of measurement time per sample. For each analysis, an energy spectrum (detected counts as a function of energy) was produced which detailed the collected sample information. All five energy spectrums were combined by computer software (OriginLab, Northampton, MA) and the average number of counts per energy channel was taken to be representative of the sample (with standard deviations). Mathematical functions were fit to the data, and the height of the energy peaks for As and Se used to derive a concentration for each of the two elements. Separate energy spectrums of 12 of the collected nail samples (randomly chosen based on identification numbers) were analyzed independently to confirm that the XRF machine and software were functioning properly (6 x 5 samples for As, and 6 x 5 samples for Se). Since the XRF technique is non-destructive, nail samples could be analyzed at Mount Allison University before being sent to Acadia University, in Wolfville, Nova Scotia, for ICP-MS analysis, which is a destructive process, making the samples no longer useful for further laboratory analyses.

Chemical analysis of nail samples at Acadia University was performed on an ELAN DRC-e Inductively Coupled Plasma-Mass Spectrometer (PerkinElmer SCIEX). Calibration of the ICP-MS was performed externally as well as internally, using element solutions acidified to 1% (v/v) HNO^3, every 8 samples. Nail samples were washed in a Branson 3510 sonicator, and were treated, sequentially with acetone (25 ml, 10 minute sonication), 3 times with deionized water (25 ml, 10 minute

sonication each), and then with acetone once more (25 ml, 10 minute sonication). Nails were digested in 1 ml HNO_3 and heated to 100°C for 40 minutes. After cooling, 1 ml of H_2O_2 was added and they were again heated to 100°C for 40 minutes. After cooling, nail samples were diluted to 20 ml (Cull, 2011). Check blanks and 5 µg/L standards, as well as 1:10 dilutions were measured every eight samples to ensure quality control.

Minimum detection limits (MDL) for XRF and ICP-MS, for As and Se, are presented in Table 5.1 (see section 2.3.3 for a discussion on ICP-MS MDL calculations). As outlined in a previous publication (Gherase & Fleming, 2011; Roy, Gherase, & Fleming, 2010), the MDL of the XRF system depends upon the size, shape, and mass, of the sample being analyzed, with higher mass samples providing a lower minimum detection limit.

Table 5.1 Minimum detection limits of arsenic and selenium for ICP-MS and XRF tests, for fingernail and toenail samples (μg/g)

	ICP-MS MDL[1] μg/g		XRF MDL[2] μg/g	
Sample	**As**	**Se**	**As**	**Se**
Fingernail	0.15	0.31	0.61 - 1.20	0.59 - 1.10
Toenail	0.15	0.31	0.61 - 1.20	0.59 - 1.10

[1]MDL: minimum detection limit

[2]Range of XRF MDL concentrations is dependent upon sample mass

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence

5.2.3 Statistical Analyses

Nail As and Se concentrations, measured by ICP-MS and XRF, were compared by examining the concordance correlation coefficient test (CCC), kappa and McNemar tests, and the Sn and Sp of the XRF method of detection relative to the ICP-MS. While the ICP-MS could be considered a gold standard test for As and Se in nails, it may not be a good gold standard because of lack of homogeneity in the sample (nail growth is time dependent, giving different estimates of As and Se exposure at different nail lengths), and the XRF is able to test the whole sample all at once. Therefore, statistical analyses incorporated comparative tests with and without a gold standard.

CCC analyses were run on As and Se concentrations that were linearly scaled to more accurately reflect the relationship between ICP-MS and XRF results (based on the reduced major axis statistical method, (Smith, 2009). The CCC test measures the distance between data points to the line y=x, however, the measurements from ICP-MS and XRF may not exhibit a 1:1 relationship. The reduced major axis comparison measures differences between detection methods where one method is scaled using the area of the triangle that encompasses the vertical and horizontal lines from the 45^{o} line. The ordinary least square method for comparing two methods does not account for errors on the x-axis (x and y axis variables are chosen arbitrarily in this analysis), and therefore assumes no measurement error in one of the detection methods. The reduced major axis analyses allow for measurement error on both axes, and is therefore more applicable for the

comparison of two methods of detection of biological data where neither is considered a good gold standard. Data were scaled by first running a CCC test to determine the line of best fit for the comparison of ICP-MS and XRF data. XRF data were multiplied by the slope of this line of best fit, and the value of its intercept was added to the product. The new scaled-XRF data were more representative of a 1:1 relationship between ICP-MS and XRF measurements. The goal of scaling the data is to more accurately represent the true relationship between the measurement methods. However, the reader should be aware that the scaling factors were created using the *existing* data, which means the scaled CCC results of correlation, while perhaps more accurate than raw CCC results, may be over-estimated. For comparative purposes, both raw and scaled CCC results are presented.

The kappa test was based on dichotomized data; fingernails and toenails were divided into categories, based on their As and Se concentrations. For As, nails that had a concentration of < 1 μg/g were labelled as "unexposed" or "test -" and nails ≥ 1.0 μg/g were labelled as "exposed" or "test +". The 1.0 μg/g cut-off value was used to reflect the maximum recommended [As] in human nail tissue (ATSDR, 2007). Nails were also divided into two categories for method comparison of [Se]. Because there is no maximum or minimum acceptable [Se] for human nails, 2.0 μg/g concentration was chosen based on having a "disease" prevalence (low Se) of approximately 80%. Concentrations of < 2.0 μg/g were labelled "exposed" or "test +" and those ≥ 2.0 μg/g were in the "unexposed" or "test -" category.

It was hypothesized that agreement between detection methods would increase as the cut-off concentration increased because the XRF did not appear to be very reliable at low concentrations of As or Se. For this reason, for each element, two higher cut-off concentrations were used. For As, cut-off values of 1.4 μg/g and 2.0 μg/g were used and concentrations of 2.6 μg/g and 3.0 μg/g were used for Se, for reasons described below. While a concentration of 1.0 μg/g of As in nail should be the cut-off limit for classifying a sample as diseased or non-diseased, by doing kappa (and sensitivity and specificity, below) analyses on separate cut-off values, it could be shown how the agreement between detection methods changes as the concentration of the sample changes.

Kappa tests return a result between -1 and 1, inclusive, and the following parameters were used to determine the degree of agreement between ICP-MS and XRF, beyond that expected due to chance alone (Dohoo, Martin & Stryhn, 2009):

- ≤ 0: negative or no agreement
- 0 – 0.20: slight agreement
- 0.21 – 0.40: fair agreement
- 0.41 – 0.60: moderate agreement
- 0.61 – 0.80: substantial agreement
- 0.81 – 1.00: almost perfect agreement

For the concordance correlation coefficients (CCC), as well as kappa, statistical analyses were run not only on all data, but also on a number of subsets of data. The first subset included dropping data for which the ICP-MS measured As or Se concentrations that were below its own minimum detection limit but for which the XRF measured a concentration that was not below its minimum detection limit. The XRF method of detection has a higher minimum detection limit (Table 5.1) than ICP-MS, and with the novel nature of the XRF, samples with results below its detection limit were deemed not reliable. Measurements by XRF were required to have a minimum photon signal strength (related to the MDL), within a defined signal window, to be deemed sufficient for use in the dataset; when sample measurements did not meet this criteria, they were not used (Knoll, 2010). With the removal of the sample result <MDL for XRF, a bias could be introduced if sample results <MDL for ICP-MS remained in the analyses. For this reason, statistical analyses were run on data with values above the MDL for ICP-MS. While the second subset analyses were intended to alleviate the detection limit bias, the bias may be over-corrected, leading to concordance correlation coefficients and kappa values that may be slightly higher than true values.

The McNemar chi-squared test was run for all kappa analyses (all samples, fingernails only and toenails only; for all cut-off values; for both As and Se) to determine if the proportions of participants categorized as positive As exposure were significantly different.

In addition to directly comparing the two tests, linear regression analysis was used to determine which factors (i.e. sample mass, sample type, water [As], survey demographic information) were associated with the discrepancy between ICP-MS and XRF measurements. In most instances, the XRF returned a larger As or Se concentration for a specific nail sample than the ICP-MS did, and this created a skewed variable when the difference was calculated. Also, for the small number of samples where the measurement by ICP-MS was greater than the measurement by XRF, the difference between them would yield a negative number. Therefore, all differences in detection method measurements were taken as the absolute value. The outcome for the regression analysis was a natural log transformation of the absolute difference between XRF and ICP-MS measurements. Regression models were assessed for normality (Shapiro-Wilk W test and visually) and homoscedasticity of residuals (Cook-Weisberg test for heteroscedasticity and visually), as well as outlying observations by running models with and without suspected outliers and examining leverage and delta-beta values.

All statistical analyses were performed using Stata 11 (StataCorp LP, College Station, Texas).

5.3 Results

5.3.1 Descriptive Statistics of Arsenic in Fingernail and Toenail Samples

A summary of the mass and number of collected samples is presented in Table 5.2 by gender and location, and in Table 5.3 by exposure group. In addition, the number of collected samples that had detectable levels of As, by both ICP-MS

and XRF methods, is described. The XRF method had a higher MDL, and therefore it was determined that the numbers of samples with detectable As were higher for the ICP-MS than the XRF (one-sided paired t-test using natural log transformed data, $p < 0.001$). Sample masses were similar across communities and exposure groups.

Table 5.2 Descriptive statistics of mass and number of fingernail and toenail samples with detectable As, by gender and location

Sample		Sample Mass (mg)			Number of samples with detectable As	
		n	**Median (SD)**	**Range**	**ICP-MS**	**XRF**
Fingernail	**Total**	147	20.6 (22.8)	0.3 - 131.8	**147**	**32**
	Hubbards	81	22.5 (24.2)	0.3 - 131.8	**81**	**18**
	Male	31	19.9 (27.8)	2.5 - 131.8	**31**	**9**
	Female	50	23.1 (21.9)	0.3 - 94.0	**50**	**9**
	Fall River	66	20.0 (21.1)	1.2 - 84.5	**66**	**14**
	Male	31	16.1 (21.0)	1.2 - 68.3	**31**	**8**
	Female	35	24.2 (21.2)	1.5 - 84.5	**35**	**6**
Toenail	**Total**	151	20.0 (31.0)	0.4 - 201.6	**151**	**26**
	Hubbards	86	25.3 (34.7)	2.8 - 201.6	**87**	**15**
	Male	36	17.3 (46.6)	5.5 - 201.6	**36**	**7**
	Female	50	25.5 (21.8)	2.8 - 88.6	**51**	**8**
	Fall River	64	18.9 (25.0)	0.4 - 100.1	**64**	**11**
	Male	31	20.1 (30.6)	1.8 – 100.1	**31**	**5**
	Female	33	18.2 (15.4)	0.4 - 73.1	**33**	**6**

Bolded rows indicate significant differences between column numbers
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence
SD: standard deviation

Table 5.3 Descriptive statistics of mass and number of fingernail and toenail samples with detectable As, by exposure group

Sample		Sample Mass (mg)			Number of samples with detectable As	
		n	**Median (SD)**	**Range**	**ICP-MS**	**XRF**
Fingernail	Total	147	20.6 (22.8)	0.3 - 131.8	**147**	**32**
	Unexposed	89	20.4 (21.9)	0.3 - 131.8	**89**	**20**
	Exposed	58	22.8 (24.4)	1.5 - 94.0	**58**	**12**
Toenail	Total	151	20.0 (31.0)	0.4 - 201.6	**151**	**26**
	Unexposed	87	21.7 (33.8)	2.2 - 201.6	**87**	**12**
	Exposed	64	16.0 (26.4)	0.4 - 100.1	**64**	**14**

Bolded rows indicate significant differences between column numbers
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence
SD: standard deviation

The [As] in fingernails and toenails, as measured by ICP-MS and XRF, are detailed in Table 5.4, by location and gender, and in Table 5.5 by exposure group. The medians and ranges for As were generally higher for the XRF than for ICP-MS, again because the XRF method had a higher MDL. A Lowess-smoothed curve plot of ICP-MS and XRF results of nail [As] is presented in Figure 5.1, and then again in Figure 5.2 with one large XRF [As] of 38.0 μg/g removed for clarity.

Table 5.4 Descriptive statistics of arsenic concentrations in fingernail and toenail samples, by gender and location

		ICP-MS (μg/g)			XRF (μg/g)		
Sample		**n**	**Mean, 95% CI**	**Range**	**n**	**Mean, 95% CI**	**Range**
Fingernail	**Total**	147	0.4 (0.3 - 0.5)	0.077* - 4.1	32	1.8 (1.4 – 2.2)	0.5 - 15.2
	Hubbards	81	0.5 (0.3 - 0.6)	0.077* - 4.1	18	1.6 (1.2 – 1.9)	0.5 - 4.1
	Male	31	0.3 (0.2 - 0.4)	0.077*- 1.3	9	1.6 (1.0 – 2.1)	0.5 - 4.1
	Female	50	0.5 (0.3 - 0.8)	0.077* - 4.1	9	1.6 (1.1 – 2.1)	0.6 - 3.3
	Fall River	66	0.3 (0.2 - 0.4)	0.077*- 2.1	14	2.0 (1.2 – 2.9)	0.6 - 15.2
	Male	31	0.4 (0.2 - 0.6)	0.077* - 2.1	8	2.6 (1.2 – 4.0)	0.7 - 15.2
	Female	35	0.3 (0.2 - 0.4)	0.077* - 1.6	6	1.2 (1.0 – 1.5)	0.6 - 4.8
Toenail	**Total**	151	0.5 (0.3 - 0.7)	0.077* - 13.2	26	1.3 (1.2 – 1.5)	0.6 – 38.0
	Hubbards	87	0.4 (0.3 - 0.5)	0.077* - 2.9	15	1.2 (1.0 – 1.5)	0.6 – 38.0
	Male	36	0.5 (0.3 - 0.6)	0.077* - 2.8	7	1.4 (0.9 – 1.8)	0.6 – 38.0
	Female	51	0.4 (0.2 - 0.6)	0.077* - 2.9	8	1.1 (0.8 – 1.3)	1.0 - 3.4
	Fall River	64	0.6 (0.2 - 1.0)	0.077* - 13.2	11	1.5 (1.2 – 1.8)	0.7 - 5.4
	Male	31	0.3 (0.2 - 0.5)	0.077* - 2.4	5	1.5 (0.7 – 2.3)	0.7 - 5.4
	Female	33	0.9 (0.0 - 1.7)	0.077* - 13.2	6	1.5 (1.1 – 1.9)	0.8 - 2.6

*Data with an asterisk are equivalent to half of the minimum detection limit of ICP-MS for each biological sample
MDLs: fingernail – 0.153 μg/g, toenail – 0.153 μg/g
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

Table 5.5 Descriptive statistics of arsenic concentrations in fingernail and toenail samples, by exposure group

		ICP-MS (μg/g)			XRF (μg/g)		
		n	**Mean, 95% CI**	**Range**	**n**	**Mean, 95% CI**	**Range**
Fingernail	**Total**	147	0.4 (0.3 - 0.5)	0.077* - 4.1	32	1.8 (1.4 – 2.2)	0.5 - 15.2
	Unexposed	89	0.2 (0.2 - 0.3)	0.077* - 3.6	20	1.7 (1.0 – 2.3)	0.5 - 13
	Exposed	58	0.7 (0.4 - 0.9)	0.077*- 4.1	12	1.9 (1.5 – 2.3)	0.6 - 15.2
Toenail	**Total**	151	0.5 (0.3 - 0.7)	0.077* - 13.2	26	1.3 (1.2 – 1.5)	0.6 - 38.0
	Unexposed	87	0.3 (0.2 - 0.4)	0.077* - 3.2	12	1.3 (1.0 – 1.6)	0.6 - 38.0
	Exposed	64	0.8 (0.3 - 1.2)	0.077* - 13.2	14	1.4 (1.2 – 1.7)	0.7 - 3.3

*Data with an asterisk are equivalent to half of the minimum detection limit of ICP-MS for each biological sample
MDLs: fingernail – 0.153 μg/g, toenail – 0.153 μg/g
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

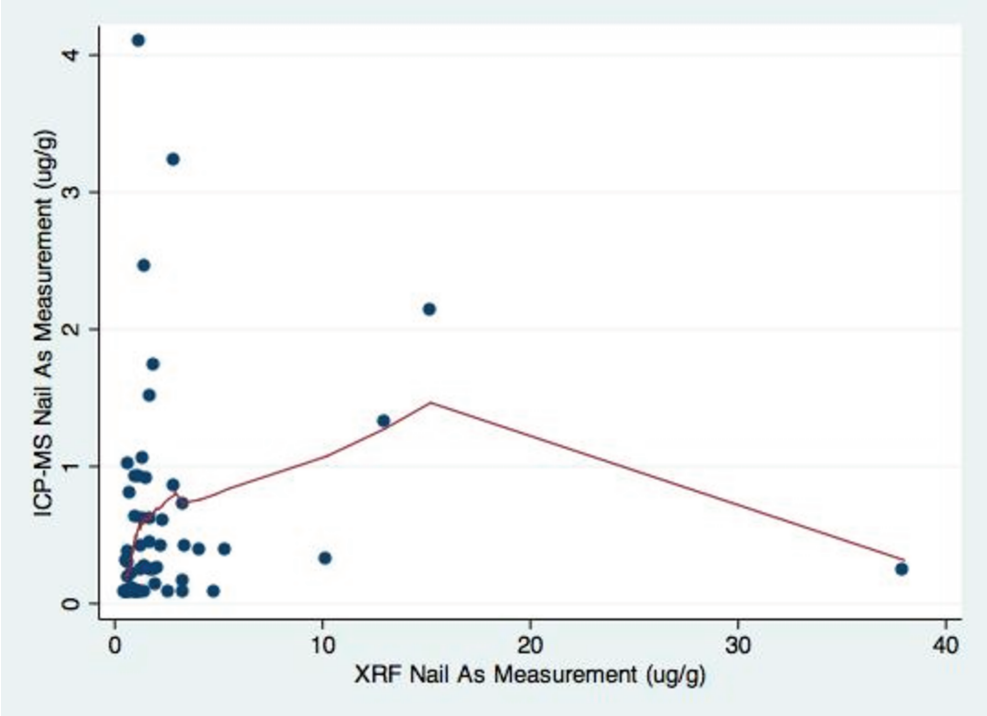

Figure 5.1 Lowess-smoothed curve plot of nail [As] measurements by ICP-MS and XRF (n=58)

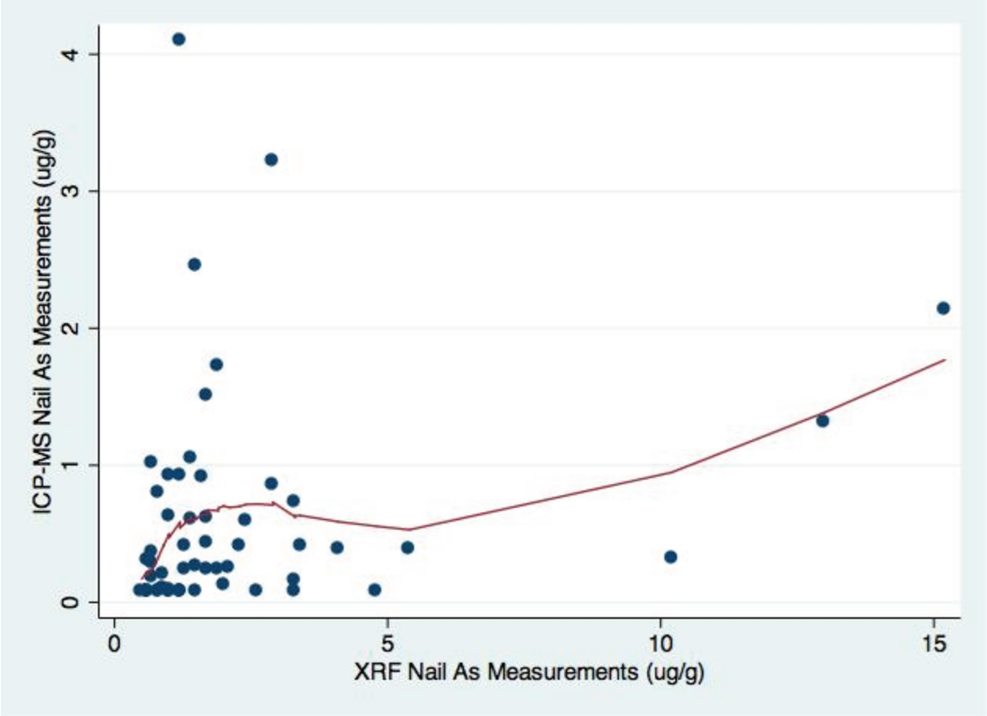

Figure 5.2 Lowess-smoothed curve plot of nail [As] measurements by ICP-MS and XRF with one large XRF measurement (38 μg/g) removed for clarity (n=57)

5.3.2 Arsenic Concentration Correlation Between ICP-MS and XRF

5.3.2.1 Concordance Correlation Coefficient Analysis

Table 5.6 shows the results of the CCC analysis of the fingernail and toenail sample [As], comparing XRF and ICP-MS results, for all samples, and then for only fingernails or only toenails. Within each of these groupings, results of subset analyses are also presented, based on restrictions in sample mass, and dropping those concentrations for which the ICP-MS method reported an [As] of less than its MDL. Additionally, scaled CCC results are also presented.

Table 5.6 Concordance correlation coefficients for natural log transformed arsenic concentration in nails as measured by ICP-MS and XRF

Sample	n	CCC	95% CI	p-value	Scaled CCC[1]	95% CI	p-value
All Samples							
All	58	**0.117**	**0.012 - 0.221**	**0.028**	**0.296**	**0.057 - 0.535**	**0.015**
>10mg	48	**0.109**	**0.010 - 0.209**	**0.032**	**0.321**	**0.062 - 0.581**	**0.015**
>20mg	37	**0.122**	**0.020 - 0.224**	**0.019**	**0.404**	**0.127 - 0.681**	**0.004**
>30mg	28	0.09	-0.025 - 0.205	0.125	0.305	-0.044 - 0.654	0.087
All, w/o MDL[2]	41	0.056	-0.093 - 0.206	0.460	0.119	-0.191 - 0.428	0.453
>10mg, w/o MDL	32	**0.094**	**-0.082 - 0.270**	**0.030**	0.191	-0.154 - 0.536	0.278
>20mg, w/o MDL	24	0.170	-0.012 - 0.352	0.067	**0.384**	**0.028 - 0.740**	**0.035**
>30mg, w/o MDL	16	0.201	-0.048 - 0.449	0.113	0.404	-0.035 - 0.842	0.071
Toenail Samples							
All	26	0.044	-0.098 - 0.186	0.545	0.124	-0.270 - 0.518	0.538
>10mg	18	0.047	-0.081 - 0.176	0.471	0.182	-0.292 - 0.656	0.452
>20mg	16	0.113	-0.027 - 0.254	0.115	**0.435**	**0.010 - 0.860**	**0.045**
>30mg	12	0.063	-0.076 - 0.201	0.373	0.287	-0.281 - 0.856	0.322
All, w/o MDL	20	-0.076	-0.247 - 0.094	0.380	0.210	-0.231 - 0.652	0.350
>10mg, w/o MDL	12	0.018	-0.210 - 0.245	0.879	0.048	-0.570 - 0.667	0.878
>20mg, w/o MDL	11	0.084	-0.183 - 0.351	0.537	0.207	-0.419 - 0.832	0.517
>30mg, w/o MDL	7	0.096	-0.324 - 0.516	0.655	0.200	-0.642 - 1.0	0.641

Bolded rows indicate significant CCCs

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

[1]Scaled CCC: data were scaled linearly using the reduced major-axis method to better reflect the relationship between ICP-MS and XRF measurements

[2]w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined arsenic concentration is greater than its own minimum detection limit (MDL)

Table 5.6 Concordance correlation coefficients for natural log transformed arsenic concentration in nails as measured by ICP-MS and XRF (continued)

Sample	n	CCC	95% CI	p-value	Scaled CCC[1]	95% CI	p-value
Fingernail Samples							
All	32	**0.166**	**0.017 - 0.315**	**0.029**	**0.401**	**0.101 - 0.701**	**0.009**
>10mg	27	0.103	-0.035 - 0.241	0.143	0.296	-0.062 - 0.654	0.105
>20mg	22	0.139	-0.006 - 0.284	0.060	**0.424**	**0.065 - 0.784**	**0.021**
>30mg	15	0.101	-0.092 - 0.294	0.306	0.285	-0.214 - 0.785	0.263
All, w/o MDL[2]	21	0.180	-0.024 - 0.384	0.084	0.331	-0.070 - 0.731	0.105
>10mg, w/o MDL	17	0.111	-0.034 - 0.257	0.134	0.160	-0.333 - 0.653	0.525
>20mg, w/o MDL	14	**0.203**	**0.006 - 0.400**	**0.043**	**0.560**	**0.172 - 0.948**	**0.005**
>30mg, w/o MDL	8	0.163	-0.117 - 0.442	0.255	0.516	-0.071 - 1.0	0.085

Bolded rows indicate significant CCCs

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

[1]Scaled CCC: data were scaled linearly using the reduced major-axis method to better reflect the relationship between ICP-MS and XRF measurements

[2]w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined arsenic concentration is greater than its own minimum detection limit (MDL)

Examining the raw data CCC results, it was found that for all available samples, the concordance correlation coefficient was 0.117 ($p = 0.028$). This coefficient improved to a maximum of 0.122 when examining only nail samples that were at least 20 mg in mass ($p = 0.019$). Dropping the <MDL ICP-MS data resulted in a maximum CCC of 0.201 for samples of at least 30 mg (not significant). Scaled data results showed marked improvement over the raw data, as expected. The CCC for all scaled data was 0.296, a 2.5-fold increase over raw data, with a maximum coefficient of 0.404 in the >30 mg nail sample category ($p = 0.015$ and $p = 0.071$, respectively). MDL truncated data showed a maximum CCC of 0.404 for samples >30 mg, equivalent to the maximum value for all scaled data.

When separating data into different nail types, it was found that the toenail only data had, overall, somewhat lower CCCs relative to all nail samples combined, with a maximum coefficient of 0.113, for toenail samples >20 mg, although these differences were not significantly different (none of the CCC differences were statistically significant, primarily due to small sample numbers – Tables 5.6). For toenails, removing <MDL ICP-MS data did not substantially improve the CCC of measurements between ICP-MS and XRF. The only increase in correlation was for toenails of >30 mg, moving from 0.063 to 0.096. Once again, as expected, scaled toenail data were much more correlated than raw toenail data. The CCC of all scaled toenail data was 0.296, a 2.8-fold increase over the unscaled raw toenail data. A maximum CCC of 0.435 was found in the ≥ 20 mg toenail mass category ($p = 0.045$). As in the raw data, scaled data without the <MCL ICP-MS values were lower

than all scaled data, with the exception of toenail samples of any mass (0.210 vs. 0.124).

Examining only fingernail samples, the concordance correlation coefficient for all samples was 0.166 ($p = 0.029$). No increasing correlation was found with increasing sample mass restriction. However, unlike with toenails, removing the <MDL ICP-MS data increased correlation somewhat between measurement methods, with a maximum correlation coefficient of 0.203 observed for <MDL truncated fingernail samples of at least 20 mg in mass ($p = 0.043$). Once again, scaled fingernail data were markedly more correlated than raw data. In comparing all available fingernail samples, the scaled concordance correlation coefficient was 2.4 times greater (0.401 vs. 0.166). The same was found for all levels of mass restriction, with the highest correlation coefficient being 0.424, for samples of at least 20 mg ($p = 0.021$). Finally, <MDL truncated scaled fingernail data were found to be the most highly correlated of all analyses, with a maximum coefficient of 0.560 found for samples of at least 20 mg ($p = 0.005$). It is important to recall that scaled CCC results, while more representative of the true correlation between ICP-MS and XRF measurements than raw CCC values, may be over-estimated.

5.3.2.2 Kappa Test

For the kappa test, results from three separate analyses are shown, based on three different XRF cut-off concentrations used to categorize samples as positive or negative: 1) 1 μg/g (ATSDR maximum contaminant limit for As in nail, (ATSDR, 2007)); 2) 1.4 μg/g (the "best" cut-off concentration, as determined by Sn and Sp

analysis, section 5.3.2.3); and 3) 2 μg/g (a high-end concentration based on data distribution). As an example for interpretation of these cut-offs, an exposed individual was one whose nail sample had an [As] of greater than or equal to 1.0 μg/g; all others were deemed unexposed. A table of positive and negative results, for all cut-off concentrations, as determined by both detection methods, is presented in Table 5.7. The results of the kappa analyses are detailed in Table 5.8, for all samples, and then for only fingernails or only toenails. Within each of these groupings, sub-groupings are again presented, based on restrictions in sample mass, and dropping those concentrations for which the ICP-MS method reported an [As] of less than its MDL.

Based on the McNemar test of homogeneity, for tests of [As], only analysis of fingernail [As] at a cut-off of 2.0 μg/g were found <u>not</u> to be significantly different in their assignment of positive values (p-value 0.096 and 0.1797, all data and <MDL ICP-MS truncated data, respectively)(Table 5.7). In all other As analysis groupings, XRF was shown to detect more positive samples than ICP-MS.

Table 5.7 Positive and negative nail arsenic classifications as measured by XRF and ICP-MS, at various cut-off concentrations of the two tests

XRF cut-off	1.0 μg/g			1.4 μg/g			2.0 μg/g		
	ICP-MS			ICP-MS			ICP-MS		
	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total
All									
XRF Negative	14	1	15	29	1	30	38	2	40
XRF Positive	35	8	43	23	5	28	16	2	18
Total	49	9	58	52	6	58	54	4	58
Toenail									
XRF Negative	6	1	7	10	2	12	17	0	17
XRF Positive	16	3	19	12	2	14	9	0	9
Total	22	4	26	22	4	26	26	0	26
Fingernail									
XRF Negative	8	0	8	17	1	18	21	2	23
XRF Positive	19	5	24	11	3	14	7	2	9
Total	27	5	32	28	4	32	28	4	32

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence

Table 5.8 Kappa statistics for agreement between XRF and ICP-MS on all nail arsenic concentrations at 3 XRF cut-off concentrations

All Samples		Kappa (95% CI)		
XRF Cut-off Concentration	**n**	**1.0 μg/g**	**1.4 μg/g**	**2.0 μg/g**
All	58	0.069	0.149	0.078
		(-0.035 - 0.173)	(-0.013 - 0.311)	(-0.124 - 0.280)
Mass >10mg	45	0.071	0.204	0.146
		(-0.064 - 0.206)	(-0.048 - 0.456)	(-0.230 - 0.522)
Mass >20mg	38	0.126	**0.370**	0.360
		(-0.057 - 0.309)	**(0.017 - 0.722)**	(-0.204 - 0.923)
Mass >30mg	27	0.163	0.348	-0.052
		(-0.111 - 0.438)	(-0.085 - 0.780)	(-0.158 - 0.055)
All, w/o MDL*	41	0.055	0.130	0.067
		(-0.077 - 0.187)	(-0.047 - 0.306)	(-0.169 - 0.303)
Mass >10mg, w/o MDL	29	0.072	0.212	0.190
		(-0.119 - 0.262)	(-0.084 - 0.508)	(-0.283 - 0.663)
Mass >20mg, w/o MDL	25	0.124	0.364	0.468
		(-0.117 - 0.365)	(-0.019 - 0.748)	(-0.131 - 1.000)
Mass >30mg, w/o MDL	15	0.224	0.333	0.000
		(-0.192 - 0.641)	(-0.166 - 0.833)	(-)

*w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined arsenic concentration is greater than its own minimum detection limit (MDL)
Bolded rows indicate significant kappa values
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

Table 5.8 Kappa statistics for agreement between XRF and ICP-MS on nail arsenic concentration at 3 XRF cut-off concentrations (continued)

Toenails		Kappa (95% CI)		
XRF Cut-off Concentration	**n**	**1.0 μg/g**	**1.4 μg/g**	**2.0 μg/g**
All	26	0.009	-0.022	0.000
		(-0.174 - 0.192)	(-0.286 - 0.241)	(-)
Mass >10mg	18	0.032	0.000	0.000
		(-0.254 - 0.319)	(-0.417 - 0.417)	(-)
Mass >20mg	16	0.072	0.091	0.000
		(-0.254 - 0.319)	(-0.424 - 0.605)	(-)
Mass >30mg	12	0.062	0.400	0.000
		(-0.414 - 0.539)	(-0.282 - 1.000)	(-)
All, w/o MDL*	20	-0.029	-0.102	0.000
		(-0.250 - 0.191)	(-0.405 - 0.202)	(-)
Mass >10mg, w/o MDL	12	0.000	-0.091	0.000
		(-0.396 - 0.396)	(-0.605 - 0.423)	(-)
Mass >20mg, w/o MDL	11	0.029	-0.038	0.000
		(-0.416 - 0.475)	(-0.609 - 0.533)	(-)
Mass >30mg, w/o MDL	7	0.087	0.300	0.000
		(-0.624 - 0.798)	(-0.467 - 1.000)	(-)

*w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined arsenic concentration is greater than its own minimum detection limit (MDL)
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

Table 5.8 Kappa statistics for agreement between XRF and ICP-MS on nail arsenic concentration at 3 XRF cut-off concentrations (continued)

Fingernails		Kappa (95% CI)		
XRF Cut-off Concentration	**n**	**1.0 μg/g**	**1.4 μg/g**	**2.0 μg/g**
All	32	0.116	0.172	0.163
		(-0.002 - 0.235)	(-0.087 - 0.432)	(-0.185 - 0.511)
Mass >10mg	27	0.100	0.200	0.182
		(-0.023 - 0.223)	(-0.141 - 0.541)	(-0.295 - 0.658)
Mass >20mg	22	0.166	0.397	0.327
		(-0.027 - 0.358)	(-0.074 - 0.868)	(-0.254 - 0.907)
Mass >30mg	15	0.237	0.189	-0.098
		(-0.068 - 0.543)	(-0.340 - 0.719)	(-0.292 - 0.097)
All, w/o MDL*	21	0.105	0.152	0.314
		(-0.029 - 0.239)	(-0.189 - 0.492)	(-0.128 - 0.756)
Mass >10mg, w/o MDL	16	0.091	0.200	0.636
		(-0.053 - 0.235)	(-0.326 - 0.726)	(-0.006 - 1.000)
Mass >20mg, w/o MDL	13	0.150	0.409	0.629
		(-0.076 - 0.375)	(-0.266 - 1.000)	(-0.021 - 1.000)
Mass >30mg, w/o MDL	7	0.222	-0.167	0.000
		(-0.196 - 0.640)	(-0.522 - 0.188)	(-)

*w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined arsenic concentration is greater than its own minimum detection limit (MDL)
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

When evaluating the kappa values for comparing [As] of all nail samples between ICP-MS and XRF measurements, at the 1 μg/g cut-off concentration, it was shown that the kappa value increased with increasing weight restrictions; the largest kappa value (0.163) was found for samples that were at least 30 mg in mass. A similar trend was found when <MDL measurements from ICP-MS were dropped from the measurements, with a maximum kappa value of 0.224 found for samples greater than or equal to 30 mg. Besides the kappa of 0.370 for all nail samples at the 1.4 μg/g cut-off, there were no statistically significant differences in kappas regardless of nail type, sample mass, or MDL restriction.

For toenail sample kappa values, agreement beyond that due to chance alone between detection methods was much poorer than it was for all samples combined. There was a similar trend, however, of increasing kappa values with increasing sample mass restriction, with a maximum value of 0.072 for samples of at least 20 mg. The 30 mg restriction category had a smaller kappa value, but it was a sample size of only 12. Similarly, with <MDL truncated data, an increasing trend was seen for kappa values and weight restriction. A maximum kappa value of 0.087 was found for samples of at least 30 mg in mass.

Kappa values for fingernail samples were, overall, higher than for toenail samples, although there were no statistically significant differences between fingernails and toenails, again likely due to small sample numbers. In general, there was again a trend of increasing kappa values with increasing mass restriction for all available fingernail samples, with an exception for the ≥ 10 mg category. The

largest kappa value (0.237) was found for fingernail samples of at least 30 mg. In contrast to all nail samples combined and toenail samples only, there was no increase in kappa value for <MDL truncated data for fingernail samples. However, there was again a positive relationship between agreement and mass restriction, with the same notable exception of the ≥ 10 mg category. The largest kappa value (0.222) was found for the ≥ 30 mg category.

Using the XRF [As] cut-off in nails that was determined to produce the best combined Sn and Sp percentage (1.4 μg/g) (section 5.3.2.3), the best kappa value for all available nail samples was found for those with a mass of at least 20 mg (0.370). Similarly, in the <MDL truncated data, the highest kappa value (0.364) was also found for samples with a mass of at least 20 mg. For all toenail samples, the largest kappa value was found for samples that were at least 30 mg in mass (0.400). In the <MDL truncated data, the largest kappa value, 0.300, was observed in the same > 30 mg category. The largest kappa value for all available fingernail samples was determined to be 0.397, for samples of at least 20 mg. In the <MDL truncated data, the largest kappa value was 0.409, observed in the sample ≥ 20mg category. Once again, for this cut-off concentration, there was a general trend of increasing kappa values with increasing mass.

Because it was hypothesized that the agreement between ICP-MS and XRF would be greater at higher [As] in nails (XRF results were generally higher than ICP-MS results – Table 5.4 and 5.5), a third round of kappa analyses were run with a XRF cut-off concentration of 2 μg/g; participants with nail concentrations of ≥ 2

μg/g of As were considered positive, all others were considered negative. The highest kappa value for all available nail samples, toenails and fingernails combined was 0.360, in the category of nails of at least 20 mg. In the <MDL truncated dataset, the largest kappa value was 0.364, observed in the same category. There were no kappa values to report for toenail samples, as there were no toenail samples for which ICP-MS determined the [As] to be ≥ 2 μg/g. For fingernails, the largest kappa value observed for all samples was 0.327, in the ≥ 20 mg category. For the <MDL truncated data, the largest kappa value, 0.636, was observed in the ≥ 10 mg category. For the kappa analyses at this cut-off concentration, there was again a trend of increasing kappa values with increasing sample mass.

5.3.2.3 Sensitivity and Specificity for Arsenic Concentrations

Table 5.9 presents the Sn and Sp for each possible cut-off value for the XRF measurements when the "true" status of the nail sample was determined to be positive or negative by ICP-MS, at a cut-off value of 1.0 μg/g (the current MCL for As in nails). Sensitivity is defined as the proportion of truly positive samples that test positive, and the Sp is defined as the proportion of truly negative samples that test negative. Table 5.9 also presents the percentage of samples correctly classified by the XRF, and the Sn and Sp of the XRF. A graph of Sn versus Sp at this 1.0 μg/g cut-off concentration is presented in Figure 5.3.

Table 5.9 Sensitivity and specificity values of XRF compared to a presumed gold standard, ICP-MS (> 1.0 μg/g cut-off), for various XRF cut-off concentrations of arsenic in 58 nail samples

Cut point (μg/g)	Sn	Sp	Correctly Classified
≥ 0.5	100.0%	0.0%	15.5%
≥ 0.6	100.0%	2.0%	17.2%
≥ 0.7	100.0%	12.2%	25.9%
≥ 0.8	88.9%	18.4%	29.3%
≥ 0.9	88.9%	24.5%	34.5%
≥ 1.0	88.9%	28.6%	37.9%
≥ 1.2	88.9%	40.8%	48.3%
≥ 1.3	77.8%	49.0%	53.5%
≥ 1.4	77.8%	53.1%	56.9%
≥ 1.5	66.7%	55.1%	56.9%
≥ 1.6	55.6%	59.2%	58.6%
≥ 1.7	55.6%	61.2%	60.3%
≥ 1.9	44.4%	67.4%	63.8%
≥ 2.0	33.3%	69.4%	63.8%
≥ 2.1	33.3%	71.4%	65.5%
≥ 2.3	33.3%	73.5%	67.2%
≥ 2.4	33.3%	75.5%	69.0%
≥ 2.6	33.3%	77.6%	70.7%
≥ 2.9	33.3%	79.6%	72.4%
≥ 3.3	22.2%	81.6%	72.4%
≥ 3.4	22.2%	87.8%	77.6%
≥ 4.1	22.2%	89.8%	79.3%
≥ 4.8	22.2%	91.8%	81.0%
≥ 5.4	22.2%	93.9%	82.8%
≥ 10.2	22.2%	95.9%	84.5%
≥ 13.0	22.2%	98.0%	86.2%
≥ 15.2	11.1%	98.0%	84.5%
≥ 38.0	0.0%	98.0%	82.8%
> 38.0	0.0%	100.0%	84.5%

Sn: Sensitivity
Sp: Specificity
XRF: x-ray fluorescence
ICP-MS: inductively coupled plasma mass spectrometry

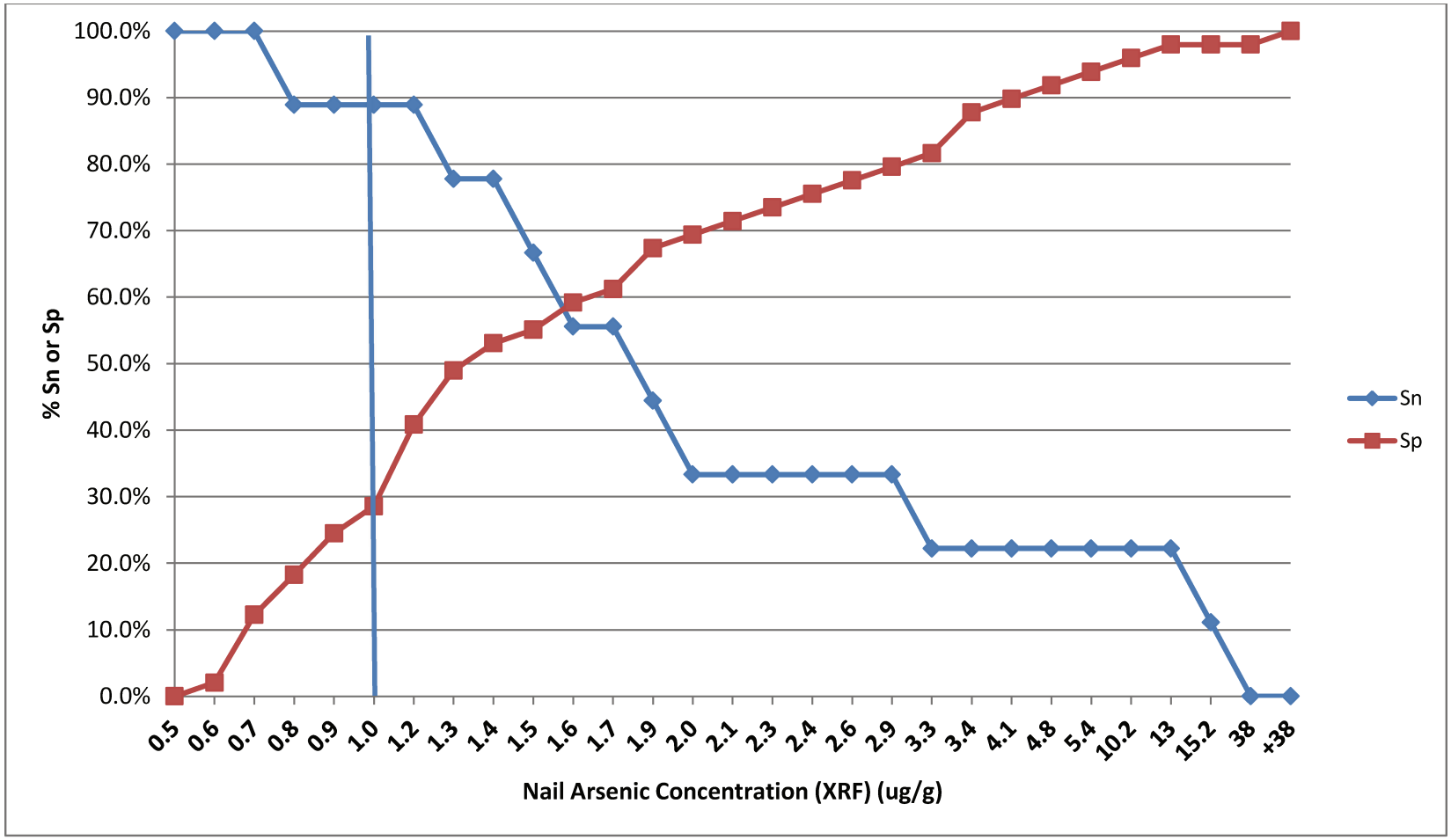

Figure 5.3 Sensitivity versus specificity plot of XRF results compared to a presumed gold standard, ICP-MS, of arsenic concentrations in 58 nail samples

Vertical line indicates XRF 1.0 μg/g cut-off

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence

Due to the difference between ICP-MS and XRF minimum detection limits, and the fact that the XRF tended to measure higher [As] in samples, positive predictive value (PPV) and negative predictive value (NPV) calculations were completed for a higher cut-off concentration of 2.0 μg/g. In addition, a cut-off concentration of 1.4 μg/g, the concentration identified as providing the largest combined Sn+Sp value in the 1.0 μg/g cut-off calculations, was investigated for PPV and NPV as well. Additionally, nail samples were analyzed separately by source (toenail or fingernail) as outlined in Table 5.10.

Table 5.10 Operating characteristics and interpretive calculations (and their 95% confidence intervals) of 3 XRF categorizations of arsenic concentrations in nail samples as compared to ICP-MS cut-off categorizations (≥ 1.0 μg/g) (n=58)

Sample (n)	XRF Cut-off Concentration (μg/g)	1.0	1.4	2.0
All (58)	Prevalence (%)	16.0 (7.3 - 27.4)	16.0 (7.3 - 27.4)	16.0 (7.3 - 27.4)
	Sensitivity (%)	88.9 (51.8 - 99.7)	66.7 (29.9 - 92.5)	33.3 (7.5 - 70.1)
	Specificity (%)	28.6 (16.6 - 43.4)	55.1 (40.2 - 69.3)	69.4 (54.6 - 81.7)
	PPV (%)	18.6 (8.4 - 33.4)	21.4 (8.3 - 41.0)	16.7 (3.6 - 41.4)
	NPV (%)	93.3 (68.1 - 99.8)	90.0 (73.5 - 97.9)	85.0 (70.2 - 94.3)
Toenails (26)	Prevalence (%)	15.0 (4.4 - 34.9)	15.0 (4.4 - 34.9)	15.0 (4.4 - 34.9)
	Sensitivity (%)	75.0 (19.4 - 99.4)	50.0 (6.8 - 93.2)	0.0 (0.0 - 60.2)
	Specificity (%)	27.3 (10.7 - 50.2)	45.5 (24.2 - 67.8)	59.1 (36.4 - 79.3)
	PPV (%)	15.8 (3.4 - 39.6)	14.3 (1.8 - 42.8)	0.0 (0.0 - 33.6)
	NPV (%)	85.7 (42.1 0 99.6)	83.3 (51.6 - 97.9)	76.5 (50.1 - 93.2)
Fingernails (32)	Prevalence (%)	16.0 (5.3 - 32.8)	16.0 (5.3 - 32.8)	16.0 (5.3 - 32.8)
	Sensitivity (%)	100.0 (47.8 - 100.0)	80.0 (28.4 - 99.5)	60.0 (14.7 - 94.7)
	Specificity (%)	29.6 (13.8 - 50.2)	63.0 (42.4 - 80.6)	77.8 (57.7 - 91.4)
	PPV (%)	20.8 (7.1 - 42.2)	28.6 (8.4 - 58.1)	33.3 (7.5 - 70.1)
	NPV (%)	100.0 (63.1 - 100.0)	94.4 (72.7 - 99.9)	91.3 (72.0 - 98.9)

PPV: positive predictive value, NPV: negative predictive value
XRF: x-ray fluorescence, ICP-MS: inductively coupled plasma-mass spectrometry

At a cut-off concentration of 1.0 μg/g, the XRF was able to correctly classify only 37.9% of all nails, with a Sn and Sp of 88.9% and 28.6% (Table 5.9), respectively. At this cut-off concentration, the PPV is low, at 18.6% due to the many false positives produced by the XRF. This means that for each nail sample the XRF classified as "positive", there was only an 18.6% chance that the sample truly was positive. On the other hand, the NPV was high, with a confidence level of 93.3% that a negatively identified nail sample by XRF truly was negative. Increasing the XRF cut-off concentration to 1.4 μg/g lowered the Sn (66.7%) but increased the Sp (55.1%) of the XRF test. The PPV and NPV at this concentration cut-off were 21.4% and 90.0%, respectively. Further increasing the cut-off concentration to 2.0 μg/g lowered the Sn again (33.3%) and also increased the Sp (69.4%), and yielded worse PPV and NPV.

Fingernail samples provided much higher Sn, Sp, PPV and NPV values than toenail samples, at each cut-off concentration. At the current MCL of 1.0 μg/g of As in nail, the XRF had 100% Sn, meaning it did not misclassify any positive samples as negative. While the Sn was extremely high, the Sp was low, 29.6%, but still higher than for all nails combined. At this concentration cut-off, the XRF also has a 100% negative predicative value for fingernails, meaning if a sample tests negative, it is 100% certain that the sample truly is negative. This is evidence that fingernails are more useful for the XRF to measure the [As] than toenail samples.

5.3.2.4 Linear Regression Analysis of the Difference in Measurements of Arsenic Concentration in Nail Samples Between XRF and ICP-MS

In the linear regression analyses to determine which factors were associated with differences in [As] measurements between XRF and ICP-MS, several variables were determined to be significant predictors of the difference between test measurements. The final model included the following variables: the mass of nail sample (and squared mass of nail sample), and sample source. Although sample source (toenail or fingernail) was not significant, it was retained in the final model because of the clear indication from CCC, kappa, and Sn/Sp analyses that XRF measurements of fingernail samples produce more reliable results compared to ICP-MS results, and therefore this relationship should be taken into account. Table 5.11 presents the multivariable linear regression analyses results for the natural log-transformed, absolute value of the difference in [As] measurements between XRF and ICP-MS, showing which factors were associated with the difference between [As] measurements by XRF and ICP-MS. The [Se] in nails or water were not significant predictors of the difference in measurements of nail [As] between ICP-MS and XRF.

Table 5.11 Multivariable linear regression analysis results for the natural log-transformed, absolute value of the difference between arsenic concentration measurements by XRF and ICP-MS (XRF minus ICP-MS) on 58 nail samples

Variable	Coef.	Robust St. Error	95% CI	p-value
Sample Mass	-0.03	0.01	-0.05 to -0.02	<0.001
(Sample Mass)^2	0.0002	0.0001	0.0001 - 0.0003	0.001
Sample Source	0.14	0.31	-0.49 - 0.76	0.660
Constant	0.91	0.29	0.33 - 1.48	0.003
n	58			
Adjusted-R^2	0.2676			

95% CI: 95% confidence interval
Sample mass: range 1 – 202 mg
Sample Source: 0 – Toenail, 1 – Fingernail
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence

Method difference regression model with back-transformed coefficients:

XRF As measurement – ICP-MS As measurement = 2.5 + [1.0*(sample mass)] + [1.00*(sample mass)^2)] + [1.15*(sample source)] + error

Sample mass was found to have a non-linear relationship with the difference in concentration measurements and was therefore included as a quadratic term. As Figure 5.4 demonstrates, for nail samples with relatively low mass, the difference between ICP-MS and XRF measurements decreases rapidly with increasing sample mass, until a sample mass of approximately 30 mg, at which point the difference between measurements levels out. The difference between measurements also was smaller in fingernails than toenails.

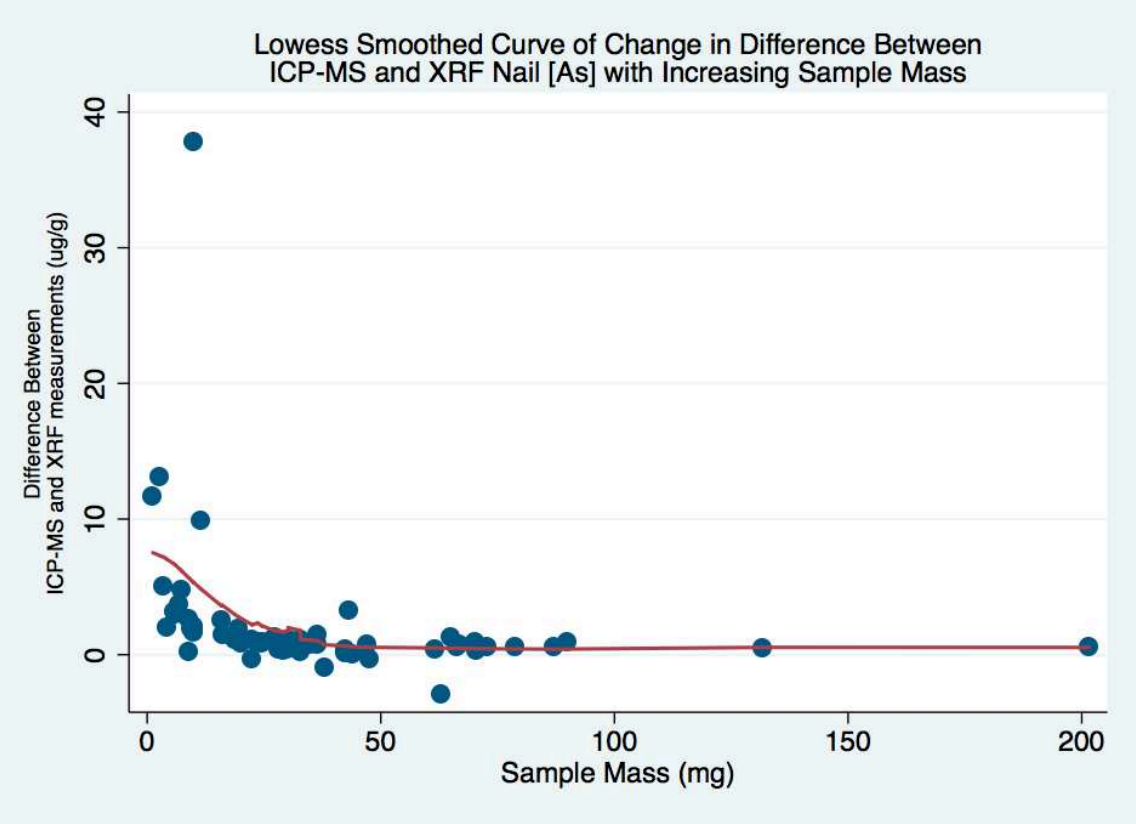

Figure 5.4 Lowess smoothed curve representing the relationship between the difference in arsenic measurements between XRF and ICP-MS and sample mass
XRF: x-ray fluorescence
ICP-MS: inductively coupled plasma-mass spectrometry

This regression model was examined before and after dropping large outlying data points (4 observations with high leverage and delta-beta values) for the difference between measurements. The XRF measurements of these 4 samples were very high, and were suspected to be erroneous because of the high concentration of lead in those samples (as measured by ICP-MS), which has an x-ray fluorescence signal very similar to As. With only 4 out of 58 samples with high lead concentrations, these 4 samples, the variable representing nail lead concentration did not reach significance in any models with homoscedastic and normally-distributed residuals. Coefficient significance and direction did not change when dropping the suspected outliers, so they remained excluded from the final model, in order to produce a model that appropriately fit the data.

A linear regression analysis, with the same predictor variables, was performed with observations limited to samples with a mass of at least 10 mg. The number of observations dropped from 58 to 43, and the adjusted-R^2 dropped from 0.2676 to 0.0651. In this model, both the linear and quadratic sample mass variables became non-significant.

5.3.3 Descriptive Statistics of Selenium in Fingernail and Toenail Samples

The number of samples with detectable [Se] is summarized in Table 5.12, by location and gender, and in Table 5.13, by exposure group. Again, the XRF method had a higher MDL than the ICP-MS, and therefore it was determined that the numbers of samples with detectable Se were higher for the ICP-MS than the XRF (one-sided paired t-test of natural log transformed data, $p = 0.027$). Sample masses

were similar across communities and exposure groups; however, numbers of samples with detectable Se were higher for the exposed groups than the unexposed groups.

Table 5.12 Descriptive statistics of mass and number of fingernail and toenail samples with detectable selenium, by gender and location

			Sample Mass (mg)		Samples with detectable [Se]	
		n	Median (SD)	Range	ICP-MS	XRF
Fingernail	**Total**	147	20.6 (22.8)	0.3 - 131.8	147	43
	Hubbards	81	22.5 (24.2)	0.3 - 131.8	81	27
	Male	31	19.9 (27.8)	2.5 - 131.8	31	9
	Female	50	23.1 (21.9)	0.3 - 94.0	50	18
	Fall River	66	20.0 (21.1)	1.2 - 84.5	66	16
	Male	31	16.1 (21.0)	1.2 - 68.3	31	8
	Female	35	24.2 (21.2)	1.5 - 84.5	35	8
Toenail	**Total**	151	20.0 (31.0)	0.4 - 201.6	150	45
	Hubbards	87	25.3 (34.7)	2.8 - 201.6	87	30
	Male	36	17.3 (46.6)	5.5 - 201.6	36	11
	Female	51	25.5 (21.8)	2.8 - 88.6	51	19
	Fall River	64	18.9 (25.0)	0.4 - 100.1	64	15
	Male	31	20.1 (30.6)	1.8 - 1001.	31	8
	Female	33	18.2 (15.4)	0.4 - 73.1	33	7

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence
SD: standard deviation

Table 5.13 Descriptive statistics of mass and number of fingernail and toenail samples, by exposure group

			Sample Mass (mg)		Samples with detectable [Se]	
		n	Median (SD)	Range	ICP-MS	XRF
Fingernail	**Total**	147	20.6 (22.8)	0.3 - 131.8	147	43
	Unexposed	89	20.4 (21.9)	0.3 - 131.8	89	29
	Exposed	58	22.8 (24.4)	1.5 - 94.0	58	14
Toenail	**Total**	151	20.0 (31.0)	0.4 - 201.6	151	45
	Unexposed	87	21.7 (33.8)	2.2 - 201.6	87	26
	Exposed	64	16.0 (26.4)	0.4 - 100.1	64	19

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence
SD: standard deviation

Fingernail and toenail [Se], as measured by ICP-MS and XRF, are presented in Table 5.14, by location and gender, and in Table 5.15, by exposure group. The minimums and medians for the XRF were slightly higher for the XRF than the ICP-MS, again likely due to the XRF method having a higher MDL. However, contrary to the As results, the upper limits of the ranges for Se were not generally higher for the XRF than for ICP-MS. A Lowess-smoothed curve plot of ICP-MS and XRF measured nail [Se] is presented in Figure 5.5, and then again in Figure 5.6 with two large XRF [Se] values (18.0 μg/g and 25 μg/g) removed for plotting clarity.

Table 5.14 Descriptive statistics of selenium concentrations in fingernail and toenail samples, by gender and location

		ICP-MS (μg/g)			XRF (μg/g)		
		n	Mean, 95% CI	Range	n	Mean, 95% CI	Range
Fingernail	**Total**	147	2.0 (1.5 – 2.6)	0.153* - 28.1	43	2.1 (0.9 – 3.2)	0.6 - 25
	Hubbards	81	2.2 (1.3 – 3.0)	0.153* - 28.1	27	2.4 (0.6 – 4.2)	0.6 - 25
	Male	31	1.7 (0.9 – 1.5)	0.153* - 9.4	9	1.6 (1.0 – 2.2)	0.8 - 1.9
	Female	50	2.5 (1.1 – 3.8)	0.153* - 28.1	18	2.8 (0.04 – 5.6)	0.6 - 25
	Fall River	66	1.8 (1.1 – 2.5)	0.153* - 16.7	16	1.4 (1.2 – 1.7)	0.7 - 18
	Male	31	1.8 (0.8 – 2.8)	0.153* - 11.7	8	1.4 (1.0 – 1.9)	0.7 - 18
	Female	35	1.8 (0.8 – 2.9)	0.153* - 16.7	8	1.5 (1.0 – 1.9)	1.0 - 1.9
Toenail	**Total**	151	2.0 (1.3 – 2.6)	0.153* - 27.3	45	1.6 (1.4 – 1.8)	0.6 - 6.2
	Hubbards	87	1.5 (1.0 – 2.1)	0.153* - 18.5	30	1.6 (1.4 – 1.9)	0.6 - 4.5
	Male	36	1.1 (0.8 – 1.4)	0.153* - 4.4	11	1.7 (1.3 – 2.1)	0.6 - 2.0
	Female	51	1.9 (1.0 – 2.7)	0.153* - 18.5	19	1.6 (1.3 – 1.9)	0.7 - 4.5
	Fall River	64	2.5 (1.3 – 3.8)	0.153* - 27.3	15	1.6 (1.2 – 1.9)	0.7 - 6.2
	Male	31	1.2 (0.7 – 1.6)	0.153* - 5.9	8	1.8 (1.2 – 2.4)	0.7 - 5.0
	Female	33	3.8 (1.4 – 6.2)	0.153*- 27.3	7	1.3 (1.0 – 1.6)	0.8 - 6.2

*Data with an asterisk are equivalent to half of the minimum detection limit of ICP-MS for each biological sample
MDLs: fingernail – 0.305 μg/g, toenail – 0.305 μg/g
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

Table 5.15 Descriptive statistics of selenium concentrations in fingernail and toenail samples, by exposure group

		ICP-MS (μg/g)			XRF (μg/g)	
		n	**Mean, 95% CI**	**Range**	**n**	**Mean, 95% CI**	**Range**
Fingernail	**Total**	147	2.0 (1.5 – 2.6)	0.153* - 28.1	43	2.1 (0.9 – 3.2)	0.6 - 25
	Unexposed	89	2.0 (1.2 – 2.7)	0.153* - 28.1	29	2.3 (0.5 – 4.1)	0.6 - 25
	Exposed	58	2.1 (1.2 – 3.0)	0.153* - 16.7	14	1.6 (1.2 – 2.0)	0.7 - 3.5
Toenail	**Total**	151	2.0 (1.3 – 2.6)	0.153* - 27.3	45	1.6 (1.4 – 1.8)	0.6 - 6.2
	Unexposed	87	1.8 (1.1 – 2.6)	0.153* - 27.3	26	1.7 (1.5 – 1.9)	0.6 - 5.0
	Exposed	64	2.1 (1.1 – 3.2)	0.153* - 25.0	19	1.5 (1.2 – 1.8)	0.8 - 6.2

*Data with an asterisk are equivalent to half of the minimum detection limit of ICP-MS for each biological sample
MDLs: fingernail – 0.305 μg/g, toenail – 0.305 μg/g
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

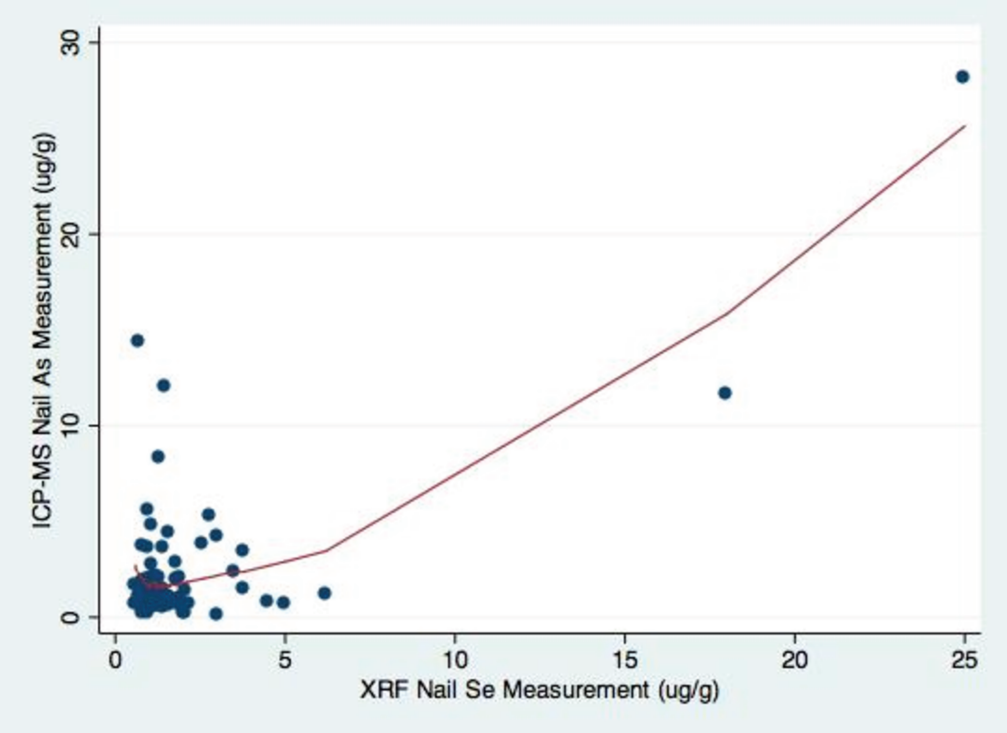

Figure 5.5 Lowess-smoothed curve plot of nail [Se] measurements by ICP-MS and XRF (n=88)

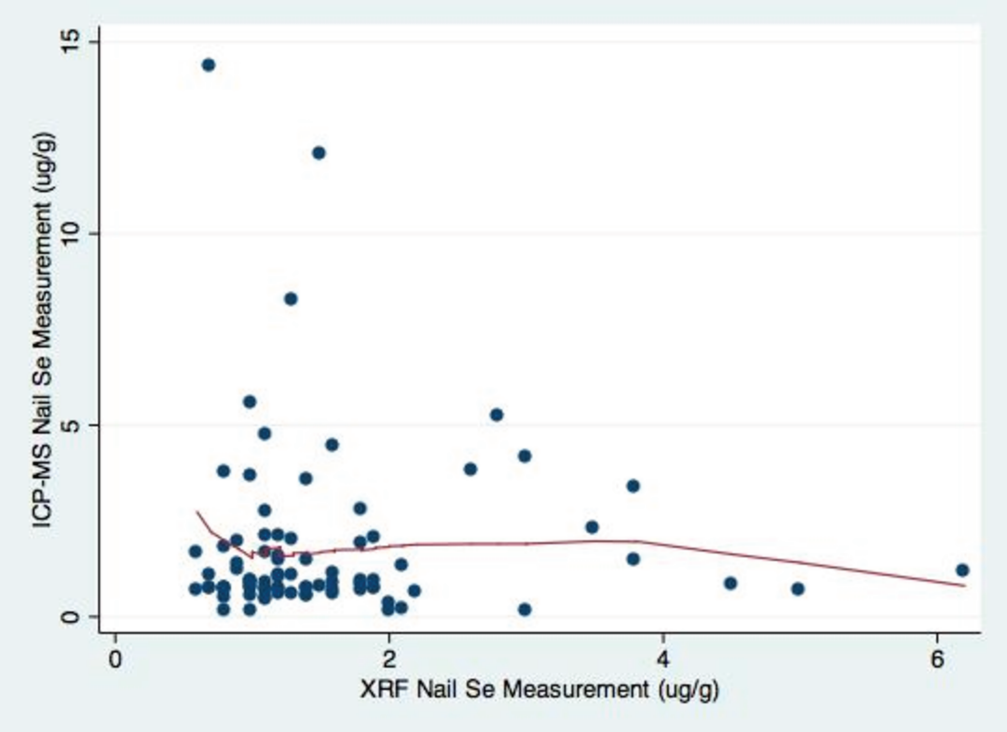

Figure 5.6 Lowess-smoothed curve plot of nail [Se] measurements by ICP-MS and XRF, with two large XRF measurements of 18 μg/g and 25 μg/g removed for clarity (n=86)

5.3.4 Selenium Concentration Correlation between ICP-MS and XRF

5.3.4.1 Concordance Correlation Coefficient Analysis

The results of CCC analysis, including 95% confidence intervals, for the measurement of [Se] in fingernail and toenail samples by ICP-MS and XRF are presented in Table 5.16. Similar to the As CCC analyses, nail samples were considered together and then separately, and were categorized by mass in each case. Additionally, CCC analyses were run on data that had been scaled linearly to more accurately reflect the relationship between measurements by ICP-MS and XRF methods.

Table 5.16 Concordance correlation coefficients for natural log transformed selenium concentration in nails as measured by ICP-MS and XRF

Sample	n	CCC	95% CI	p-value	Scaled CCC[1]	95% CI	p-value
All Samples							
All	86	**0.258**	**0.085 - 0.432**	**0.004**	**0.295**	**0.100 - 0.490**	**0.003**
>10mg	73	**0.277**	**0.101 - 0.452**	**0.002**	**0.333**	**0.126 - 0.539**	**0.002**
>20mg	58	**0.274**	**0.086 - 0.463**	**0.004**	**0.345**	**0.114 - 0.576**	**0.003**
>30mg	42	0.045	-0.097 - 0.186	0.536	0.097	-0.210 - 0.404	0.534
All, w/o MDL[2]	82	**0.321**	**0.139 - 0.503**	**0.001**	**0.345**	**0.152 - 0.538**	**< 0.001**
>10mg, w/o MDL	70	**0.319**	**0.143 - 0.503**	**0.001**	**0.363**	**0.157 - 0.569**	**0.001**
>20mg, w/o MDL	55	**0.334**	**0.141 - 0.528**	**0.001**	**0.405**	**0.179 - 0.630**	**< 0.001**
>30mg, w/o MDL	39	0.117	-0.019 - 0.252	0.093	0.270	-0.028 - 0.569	0.076
Toenail Samples							
All	45	-0.051	-0.296 - 0.193	0.681	0.063	-0.235 - 0.360	0.680
>10mg	32	-0.115	-0.354 - 0.125	0.347	0.168	-0.180 - 0.516	0.344
>20mg	27	0.025	-0.222 - 0.272	0.844	0.039	-0.352 - 0.431	0.844
>30mg	23	0.146	-0.046 - 0.338	0.135	0.312	-0.074 - 0.698	0.113
All, w/o MDL	44	0.009	-0.251 - 0.268	0.948	0.010	-0.292 - 0.312	0.948
>10mg, w/o MDL	32	-0.115	-0.354 - 0.125	0.347	0.168	-0.180 - 0.516	0.344
>20mg, w/o MDL	27	0.025	-0.222 - 0.272	0.844	0.039	-0.352 - 0.431	0.844
>30mg, w/o MDL	23	0.146	-0.045 - 0.338	0.135	0.312	-0.074 - 0.698	0.113

[1]Scaled CCC: data were scaled linearly using the reduced major-axis method to better reflect the relationship between ICP-MS and XRF measurements

[2]w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined selenium concentration is greater than its own minimum detection limit (MDL)

Bolded rows indicate significant CCCs

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

Table 5.16 Concordance correlation coefficients for natural log transformed selenium concentration in nails as measured by ICP-MS and XRF (continued)

Sample	n	CCC	95% CI	p-value	Scaled CCC[1]	95% CI	p-value
Fingernail Samples							
All	41	**0.500**	**0.294 - 0.706**	**< 0.001**	**0.548**	**0.331 - 0.765**	**< 0.001**
>10mg	39	**0.396**	**0.170 - 0.623**	**0.001**	**0.461**	**0.207 - 0.715**	**< 0.001**
>20mg	28	**0.376**	**0.101 - 0.651**	**0.007**	**0.438**	**0.127 - 0.748**	**0.006**
>30mg	18	-0.088	-0.322 - 0.146	0.460	0.181	-0.293 - 0.655	0.454
All, w/o MDL[2]	39	**0.594**	**0.392 - 0.793**	**< 0.001**	**0.611**	**0.409 - 0.813**	**< 0.001**
>10mg, w/o MDL	36	**0.498**	**0.267 - 0.729**	**< 0.001**	**0.533**	**0.292 - 0.773**	**< 0.001**
>20mg, w/o MDL	25	**0.505**	**0.234 - 0.777**	**< 0.001**	**0.559**	**0.277 - 0.840**	**< 0.001**
>30mg, w/o MDL	15	0.011	-0.222 - 0.244	0.927	0.025	-0.518 - 0.569	0.927

[1]Scaled CCC: data were scaled linearly using the reduced major-axis method to better reflect the relationship between ICP-MS and XRF measurement

[2]w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined selenium concentration is greater than its own minimum detection limit (MDL)

Bolded rows indicate significant CCCs

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence, 95% CI: 95% confidence interval

For all nail samples combined, the largest CCC value obtained was 0.277, for all samples of at least 10 mg in mass ($p = 0.002$). For these analyses, in contrast to the As analyses, no discernible relationship existed between sample mass and agreement. For <MDL truncated data, the largest CCC value (0.334, $p = 0.001$) was also found for samples of at least 20 mg. Scaled data showed marked improvement over raw data, with the largest value (0.345, $p < 0.001$) found for samples of at least 20 mg. For <MDL truncated data, a maximum CCC value of 0.405, again, was found for the ≥ 20 mg group ($p < 0.001$).

Correlation between toenail samples only was quite poor in relation to all nails combined. Many analyses found a negative CCC, and the largest CCC value found was 0.146, for the >=30 mg category (not significant). No samples were <MDL on ICP-MS so no data was dropped in these analyses. Scaled data showed marked improvement over raw data, with the largest CCC reaching 0.312 for samples of at least 30 mg (not significant).

CCC analysis for all available fingernail samples showed a CCC of 0.500 ($p < 0.001$), and CCC values dropped as mass restriction increased. Dropping <MDL ICP-MS data improved correlation between the two detection methods in each separate analysis, with a maximum CCC value of 0.594 observed for all samples ($p < 0.001$). Scaling fingernail data improved CCC values even further, with coefficients of 0.548 and 0.611 for all samples in the raw data and <MDL truncated data, respectively ($p < 0.001$ for both). No discernible relationship between correlation and mass of sample was observed.

5.3.4.2 Kappa Tests

For every grouping of Se analysis, the McNemar test showed that detection methods did not differ significantly in their assignment of positive values (range of p-values from 0.1025 to 1.000). Based on data distribution, a [Se] cut-off of 2.0 μg/g or more was used to create a dichotomous outcome for the kappas. For each kappa cut-off concentration, tables are presented for the number of nail samples categorized as positive or negative by XRF and ICP-MS (Table 5.17). Kappa values for analysis of all cut-off concentrations are presented in Table 5.18, along with 95% confidence interval, categorized by nail type and mass, as well as for all nail samples or only those that were above the minimum detection limit for ICP-MS.

Table 5.17 Positive and negative nail selenium classifications as measured by XRF and ICP-MS, at various cut-off concentrations of the two tests

	2.0 μg/g			2.6 μg/g			3.0 μg/g		
		ICP-MS			ICP-MS			ICP-MS	
	Negative	Positive	Total	Negative	Positive	Total	Negative	Positive	Total
All									
XRF Negative	55	16	71	65	12	77	67	11	78
XRF Positive	10	7	17	6	5	11	6	4	10
Total	65	23	88	71	17	88	73	15	88
Toenail									
XRF Negative	26	8	34	31	7	38	32	7	39
XRF Positive	8	3	11	5	2	7	5	1	6
Total	34	11	45	36	9	45	37	8	45
Fingernail									
XRF Negative	29	8	37	34	5	39	35	4	39
XRF Positive	2	4	6	1	3	4	1	3	4
Total	31	12	43	35	8	43	36	7	43

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence

Table 5.18 Kappa statistics for agreement between XRF and ICP-MS on all nail selenium concentrations at 3 XRF cut-off concentrations

All Samples		Kappa (95% CI)		
Cut-off Concentration	**n**	**2.0 μg/g**	**2.6 μg/g**	**3.0 μg/g**
All	88	0.180	0.242	0.213
		(-0.047 - 0.407)	(-0.008 - 0.492)	(-0.045 - 0.471)
Mass >10mg	75	0.148	0.198	0.238
		(-0.081 - 0.377)	(-0.069 - 0.465)	(-0.064 - 0.540)
Mass >20mg	60	0.030	0.140	0.156
		(-0.160 - 0.221)	(-0.108 - 0.389)	(-0.117 - 0.429)
Mass >30mg	43	-0.045	0.000	0.000
		(-0.131 - -0.042)	(-)	(-)
All, w/o MDL	84	0.211	**0.259**	0.230
		(-0.020 - 0.441)	**(0.006 - 0.512)**	(-0.032 - 0.493)
Mass >10mg, w/o MDL	72	0.171	0.196	0.236
		(-0.057 - 0.398)	(-0.071 - 0.462)	(-0.066 - 0.537)
Mass >20mg, w/o MDL	57	0.059	0.139	0.155
		(-0.125 - 0.244)	(-0.107 - 0.385)	(-0.116 - 0.426)
Mass >30mg, w/o MDL	40	0.000	0.000	0.000
		(-)	(-)	(-)

*w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined selenium concentration is greater than its own minimum detection limit (MDL)

Bolded rows indicate significant kappas

ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence

95% CI: 95% confidence interval

Table 5.18 Kappa statistics for agreement between XRF and ICP-MS on all nail selenium concentrations at 3 XRF cut-off concentrations (continued)

Toenail Samples		Kappa (95%CI)		
Cut-off Concentration	**n**	**2.0 μg/g**	**2.6 μg/g**	**3.0 μg/g**
All	45	0.037	0.091	-0.011
		(-0.262 - 0.337)	(-0.228 - 0.410)	(-0.293 - 0.271)
Mass >10mg	34	-0.055	0.000	0.000
		(-0.162 - 0.051)	(-)	(-)
Mass >20mg	29	0.000	0.000	0.000
		(-)	(-)	(-)
Mass >30mg	24	0.000	0.000	0.000
		(-)	(-)	(-)
All, w/o MDL	44	0.063	0.123	0.016
		(-0.244 - 0.369)	(-0.205 - 0.451)	(-0.278 - 0.310)
Mass >10mg, w/o MDL	34	-0.055	0.000	0.000
		(-0.162 - 0.051)	(-)	(-)
Mass >20mg, w/o MDL	29	0.000	0.000	0.000
		(-)	(-)	(-)
Mass >30mg, w/o MDL	24	0.000	0.000	0.000
		(-)	(-)	(-)

*w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined selenium concentration is greater than its own minimum detection limit (MDL)
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence
95% CI: 95% confidence interval

Table 5.18 Kappa statistics for agreement between XRF and ICP-MS on all nail selenium concentrations at 3 XRF cut-off concentrations (continued)

Fingernail Samples		Kappa (95% CI)		
Cut-off Concentration	**n**	**2.0 μg/g**	**2.6 μg/g**	**3.0 μg/g**
All	43	**0.354**	**0.429**	**0.484**
		(0.030 - 0.677)	**(0.063 - 0.796)**	**(0.105 - 0.864)**
Mass >10mg	41	0.283	0.332	0.384
		(-0.054 - 0.621)	(-0.063 - 0.726)	(-0.038 - 0.806)
Mass >20mg	31	0.075	0.295	0.295
		(-0.276 - 0.425)	(-0.162 - 0.753)	(-0.162 - 0.753)
Mass >30mg	19	-0.092	0.000	0.000
		(-0.268 - 0.085)	(-)	(-)
All, w/o MDL	40	**0.396**	**0.423**	**0.470**
		(0.077 - 0.715)	**(0.055 - 0.791)**	**(0.097 - 0.861)**
Mass >10mg, w/o MDL	38	0.327	0.325	0.379
		(-0.007 - 0.662)	(-0.070 - 0.721)	(-0.044 - 0.803)
Mass >20mg, w/o MDL	28	0.125	0.291	0.291
		(-0.228 - 0.478)	(-0.162 - 0.745)	(-0.162 - 0.745)
Mass >30mg, w/o MDL	16	0.000	0.000	0.000
		(-)	(-)	(-)

*w/o MDL: subset of data which includes only nail samples for which the ICP-MS determined selenium concentration is greater than its own minimum detection limit (MDL)
Bolded rows indicate significant kappas
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence
95% CI: 95% confidence interval

For the kappa analysis of all available nail samples of [Se], the highest level of agreement was found for all samples in the 3.0 μg/g cut-off category (0.484). In general, a trend of decreasing agreement with increasing sample mass was observed across all analyses. There were no statistically significant differences in kappas regardless of nail type, sample mass, or MDL restriction.

For the analyses at the 2.0 μg/g cut-off, the largest kappa value for all available data was 0.180, including all sample masses. When considering <MDL truncated data, the highest kappa value (0.211) was again found for all sample masses.

Toenail kappa analyses were overall very poor, with the largest kappa values of 0.037 and 0.063 found for samples of all masses, for all data and <MDL truncated data, respectively. Fingernail samples showed markedly better agreement than toenail samples. The largest kappa value was found for samples of all masses for all data (0.354) and for <MDL truncated data (0.396).

For the best cut-off concentration as determined by Sn and Sp calculations, 2.6 μg/g, the largest kappa value for all nails was observed for samples of all masses (0.242). The highest kappa value for <MDL truncated data, 0.259, was also observed for all available nail samples. In most instances, there were no toenail samples that were determined to be greater than 2.6 μg/g by XRF. Therefore, only analyses that included all available samples produced kappa values. The largest, and only, kappa value for all available toenail samples was 0.091, and 0.123 for the <MDL truncated data. Kappa analysis of fingernail samples determined that the

highest kappa value for all samples was 0.429, for all sample masses. For <MDL truncated data, the largest kappa value found was 0.423, again for all samples masses.

Overall, the kappa values for all nail samples, with a cut-off value of 3 μg/g, were numerically but not statistically significantly higher than for the lower cut-off values. In this case, the largest kappa value was found for nail samples of at least 10 mg (0.238). For <MDL truncated data, a kappa value of 0.236 was observed for the same mass category. Once again, detection methods showed very low agreement for [Se] in toenail samples. For all categories with mass restrictions, kappa analyses were not possible due to lack of samples ≥ 3 μg/g, as measured by ICP-MS, which also had a detectible [Se] by XRF. A negative agreement of -0.011 was found for all toenail samples, and a very small agreement of 0.016 was found for all <MDL truncated toenail samples. Fingernail samples had markedly improved agreement over toenail samples. The largest kappa value for all samples was 0.484, and 0.470 for the <MDL truncated data.

5.3.4.3 Sensitivity and Specificity for Selenium Concentrations

The ability of the XRF to correctly classify a nail sample as exposed (test +) or unexposed (test -), based on [Se] of various cut-off levels, was compared to ICP-MS quantification of [Se] in nail samples. In this analysis, a positive sample was a nail sample that the ICP-MS determined to have **less** than 2.0 μg/g of Se, and a negative sample was one that had **at least** 2.0 μg/g of Se. Table 5.19 presents the Sn and Sp

for each possible cut-off concentration for the XRF, as well as the percentage of samples correctly classified. A Sn versus Sp plot is presented in Figure 5.7.

Table 5.19 Sensitivity and specificity values of XRF compared to a presumed gold standard, ICP-MS (< 2.0 μg/g cut-off), for various XRF cut-off concentrations of selenium in 88 nail samples

Cut point	Sn	Sp	Correctly Classified
≤ 0.6	0.0%	100.0%	74.4%
≤ 0.7	3.1%	100.0%	72.1%
≤ 0.8	7.8%	95.5%	69.8%
≤ 0.9	18.8%	90.9%	62.8%
≤ 1.0	23.4%	90.9%	59.3%
≤ 1.1	34.4%	81.8%	53.5%
≤ 1.2	43.8%	68.2%	50.0%
≤ 1.3	54.7%	63.6%	43.0%
≤ 1.4	57.8%	54.6%	43.0%
≤ 1.5	65.6%	50.0%	38.4%
≤ 1.6	67.2%	45.5%	38.4%
≤ 1.8	73.4%	40.9%	34.9%
≤ 1.9	79.7%	36.4%	31.4%
≤ 2.0	84.4%	31.8%	29.1%
≤ 2.1	87.5%	31.8%	26.7%
≤ 2.2	90.6%	31.8%	24.4%
≤ 2.6	92.2%	31.8%	23.3%
≤ 2.8	92.2%	27.3%	24.4%
≤ 3.0	92.2%	22.7%	25.6%
≤ 3.5	93.8%	18.2%	25.6%
≤ 3.8	93.8%	13.6%	26.7%
≤ 4.5	95.3%	9.1%	26.7%
≤ 5.0	96.9%	9.1%	25.6%
≤ 6.2	98.4%	9.1%	24.4%
≤ 18.0	100.0%	9.1%	23.3%
≤ 25.0	100.0%	4.6%	24.4%
< 25.0	100.0%	0.0%	25.6%

Sn: sensitivity
Sp: specificity
XRF: x-ray fluorescence
ICP-MS: inductively coupled plasma-mass spectrometry

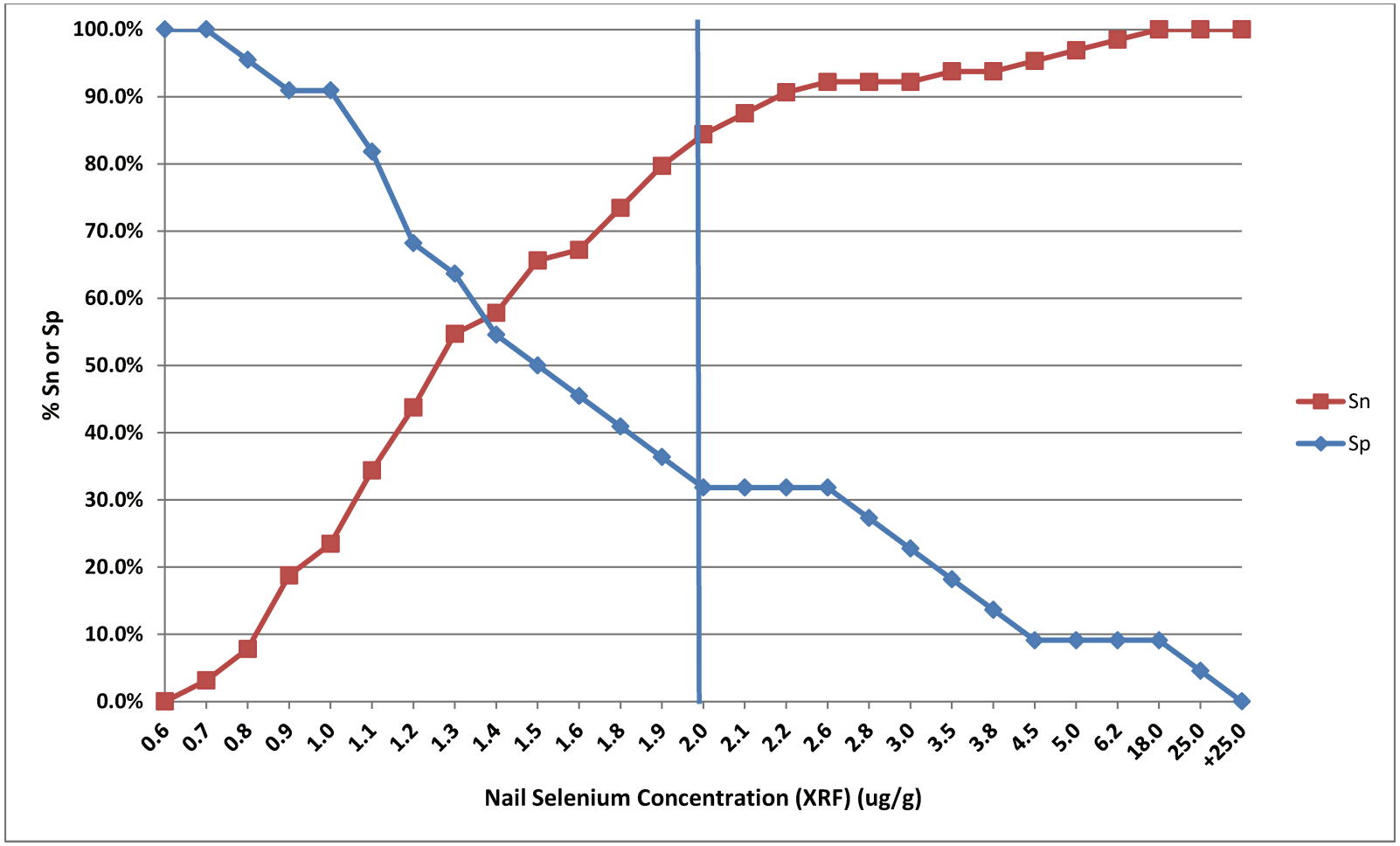

Figure 5.7 Sensitivity versus specificity plot of XRF results compared to a presumed gold standard, ICP-MS, of selenium concentrations in 88 nail samples

Vertical line indicates XRF 2.0 μg/g cut-off

XRF: x-ray fluorescence, ICP-MS: inductively coupled plasma-mass spectrometry

Due to the difference between ICP-MS and XRF minimum detection limits, and the fact that the XRF tended to measure higher [Se] in nails, PPV and NPV calculations were completed for a higher cut-off concentration of 3.0 μg/g of Se in nails. In addition, a cut-off concentration of 2.6 μg/g, the concentration identified as providing the largest combined Sn+Sp value in the 2.0 μg/g cut-off calculations, was investigated for PPV and NPV as well, as outlined in Table 5.20.

Table 5.20 Operating characteristics and interpretive calculations (and their 95% confidence intervals) of 3 XRF categorizations of selenium concentrations in nail samples as compared to ICP-MS cut-off categorizations (≤ 2.0 μg/g) (n=88)

Sample (n)	Cut-off Concentration (μg/g)	2.0	2.6	3.0
All (88)	Prevalence (%)	74.0 (64.0 - 83.2)	74.0 (64.0 - 83.2)	74.0 (64.0 - 83.2)
	Sensitivity (%)	84.4 (73.1 - 92.2)	92.2 (82.7 - 97.4)	92.2 (82.7 - 97.4)
	Specificity (%)	31.8 (13.9 - 54.9)	27.3 (10.7 - 50.2)	22.7 (7.8 - 45.4)
	PPV (%)	78.3 (66.7 - 87.3)	78.7 (67.7 - 87.3)	77.6 (66.6 - 86.4)
	NPV (%)	41.2 (18.4 - 67.1)	54.5 (23.4 - 83.3)	50.0 (18.7 - 81.3)
Toenails (45)	Prevalence (%)	75.0 (60.0 - 86.8)	75.0 (60.0 - 86.8)	75.0 (60.0 - 86.8)
	Sensitivity (%)	75.8 (57.7 - 88.9)	84.8 (68.1 - 94.9)	84.8 (68.1 - 94.4)
	Specificity (%)	27.3 (6.0 - 61.0)	18.2 (2.3 - 51.8)	9.1 (0.2 - 41.4)
	PPV (%)	75.8 (57.5 - 88.9)	75.7 (58.8 - 88.2)	73.7 (56.9 - 86.6)
	NPV (%)	27.3 (6.0 - 61.0)	28.6 (3.4 - 71.0)	16.7 (0.4 - 64.1)
Fingernails (43)	Prevalence (%)	74.0 (58.0 - 86.1)	74.0 (58.0 - 86.1)	74.0 (58.0 - 86.1)
	Sensitivity (%)	93.5 (78.6 - 99.2)	100.0 (88.8 - 100.0)	100.0 (88.8 - 100.0)
	Specificity (%)	36.4 (10.9 - 69.2)	36.4 (10.9 - 69.2)	36.4 (10.9 - 69.2)
	PPV (%)	80.6 (64.0 - 91.8)	81.6 (65.7 - 92.3)	81.6 (65.7 - 92.3)
	NPV (%)	66.7 (22.3 - 95.7)	100.0 (39.8 - 100.0)	100.0 (39.8 - 100.)

PPV: positive predictive value
NPV: negative predictive value

The Sn of the XRF at the 2.0 μg/g concentration was 84.4% and its Sp was 31.8% (total 116.2%). This means that at a true nail Se concentration of 2.0 μg/g, the XRF was able to correctly identify a truly positive sample as such 84.4% of the time, and was able to correctly identify a truly negative sample 31.8% of the time. At this concentration, if the XRF classified a sample as positive, it was 78.3% certain that the sample truly was positive (PPV). Likewise, if a sample tested negative, it was 41.2% certain that the sample truly was negative (NPV). As shown by Table 5.19, the 2.6 μg/g cut-off concentration provided the highest combined Sn and Sp value of the three cut-off concentrations investigated (119.5%). At 2.6 μg/g, both the Sn and the Sp had the highest values observed for the XRF (92.2% and 27.3%, respectively). At this cut-off concentration, the PPV increased only marginally, by 0.5%, but the NPV increased by 13.3% to 54.5%. The 3.0 μg/g cut-off concentration had the same Sn as the previous cut-off but the Sp decreased slightly, by 4.6%, to 22.7%. The 3.0 μg/g cut-off had the lowest PPV observed, 77.6%, but had the second highest NPV, 50.0%.

Fingernails were found to have higher Sn and Sp values than toenails, at each cut-off concentration. At both the 2.6 μg/g and 3.0 μg/g cut-off concentrations, the XRF showed 100% Sn and an increased Sp over all nails combined, 36.4% for both. With a Sn of 100%, the XRF was able to correctly identify all positive samples and did not misclassify any as negative. As well, the XRF had a negative predictive value of 100% at both cut-offs, a substantial increase over the 54.5% and 50.0% NPV for all nails at the 2.6 μg/g and 3.0 μg/g cut-offs, respectively. With this NPV, if the XRF

gives a negative test result, it is 100% certain that the sample truly is negative. This is strong evidence that fingernail samples are superior to toenail samples for use by the XRF to determine the [Se] in nails.

5.3.4.4 Linear Regression Analysis of the Difference in Concentration of Selenium in Nail Samples Measured by XRF and ICP-MS

Table 5.21 presents univariable regression analyses for the absolute, natural log transformed difference in [Se] measurements between XRF and ICP-MS. There were no combinations of potential predictors that led to a suitable multiple linear regression model with homoscedastic and normally distributed residuals, therefore, only univariable analyses are presented. Univariable models also did not produce homoscedastic or normally distributed residuals, and therefore interpretation of coefficients is limited.

Table 5.21 Univariable linear regression analysis results for the scaled difference between selenium concentration measurements by XRF and ICP-MS (XRF minus ICP-MS) on 88 nail samples

Variable	Coef.	Robust St. Error	p-value	95% CI
Nail [As]	0.58	0.24	0.019	0.10 – 1.09
Sample Mass*	-0.02	0.01	0.016	-0.04 to -0.004
(Sample Mass)²*	0.0001	0.0001	0.190	-0.0001 – 0.0003
Sample Source	-0.18	0.33	0.580	-0.83 – 0.47

*Sample mass & (sample mass)² included in single model
Nail [As]: range 0 – 13 μg/g (measured by ICP-MS)
Sample mass: range 0.3 – 202 mg
Water [As]: range <MDL – 309 μg/L
ICP-MS: inductively coupled plasma-mass spectrometry, XRF: x-ray fluorescence
95% CI: 95% confidence interval
Constant ranged from -0.69 to -0.37 for the above univariable analyses.

The mass of the nail sample being analyzed was significantly associated with the difference between measurements of [Se]. The association between sample mass and measurement difference was non-linear and was modeled using a quadratic version of the sample mass variable. The difference between measurements becomes smaller as the sample mass increases until approximately 30 mg. Overall, the greatest decrease (and overall change) in measurement differences occurs between 0 and 20 mg. A Lowess smoothed curve is presented in Figure 5.8 representing the relationship between sample mass and measurement differences.

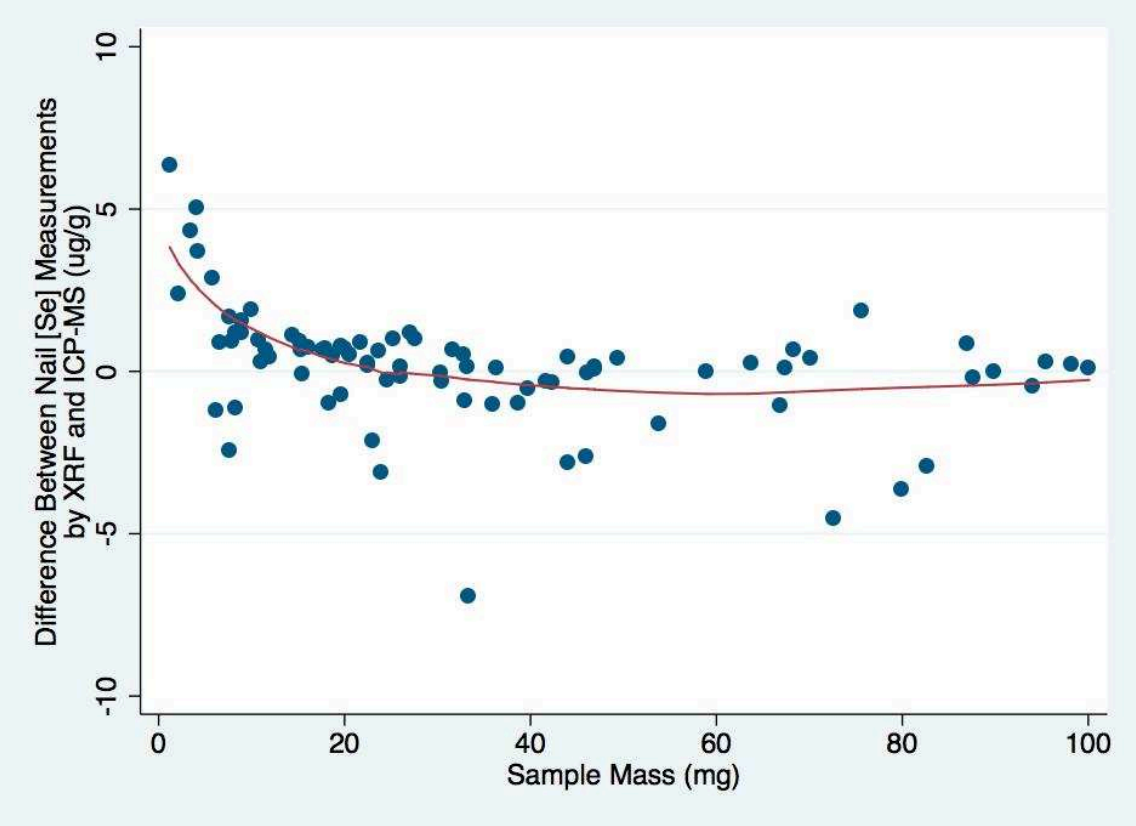

Figure 5.8 Lowess smoothed curve representing the relationship between the difference in selenium measurements between XRF and ICP-MS and sample mass
XRF: x-ray fluorescence
ICP-MS: inductively coupled plasma-mass spectrometry

The source of the nail sample analyzed (toenail or fingernail) was not found to be significantly associated with the difference between XRF and ICP-MS [Se] measurements. Nail [As] was negatively associated with the difference between nail [Se] measurements by ICP-MS and XRF ($p < 0.001$), meaning that as the concentration of As in a nail sample increased, the difference in [Se] in the same sample, as measured by each method, decreased. It should also be noted that nail [As] was a strong predictor of nail [Se] in linear regression analyses (results not shown) and that a Lowess smoothed-curve plot shows a roughly linear relationship between both values, though there is considerable variation around the line of best fit (not shown).

5.4 Discussion

Several factors were found to be associated with the relationship between As and Se measurements in nail samples made by ICP-MS and XRF, including: fingernails versus toenails, and mass of the sample. However, adjusting for those factors did not substantially improve the low results in overall CCC, kappa, and Sp values into ranges that would currently make the XRF a suitable replacement for ICP-MS..

Fingernail and toenail samples ranged in [As] from less than the ICP-MS detection limit for fingernail (0.153 μg/g) to 4.1 μg/g and from less than the ICP-MS detection limit for toenails (0.153 μg/g) to 13.2 μg/g (Table 5.4). For Se, fingernail concentrations ranged from less than the ICP-MS detection limit for fingernails (0.305 μg/g) to 28.3 μg/g, and toenail [Se] ranged from less than the

detection limit for toenails (0.305 μg/g) to 27.1 μg/g (Table 5.14). These As and Se concentrations in fingernail and toenail are within the ranges of other non-exposed and exposed populations (Gault et al., 2008; Pearce et al., 2010).

The CCC, kappa, and Sn and Sp analyses for comparing ICP-MS and XRF As and Se concentrations consistently revealed higher numerical relationships in fingernail than toenail samples (applies for both scaled and unscaled CCC results). Although 95% confidence intervals (CI) for the largest fingernail and toenail sample CCC values overlapped for [As] (raw and scaled) (Table 5.6) and scaled Se CCC (Table 5.16), the 95% CIs of raw Se CCC values did not overlap, showing statistically significant differences (toenail: CCC 0.146, 95% CI -0.046 – 0.338; fingernail CCC 0.594, 95% CI 0.392 – 0.793). Even without statistical significance for the other 5 analyses (raw and scaled toenail As CCC, raw and scaled fingernail As CCC, and scaled toenail Se CCC), this result provides support for choosing fingernail samples over toenail samples for determining As or Se concentration by XRF.

Sensitivity and specificity analyses to assess the ability of the XRF to correctly identify a positive or negative sample determined that the XRF had relatively high Sn but its Sp was quite low (Tables 5.10 and 5.20). Across three cut-off concentrations for each element, it was found that, if the XRF were to be used in the field, it would be best suited as a screening tool, as opposed to a confirmatory one. However, on the whole, the XRF results, both on a continuous and dichotomous scale, were not accurate when compared to ICP-MS. Additional work is needed to

fine-tune the XRF before it could be considered an alternative to the ICP-MS. To address some of the errors in measurement by XRF, several steps could be taken. In the detection of emitted photons from the sample, two or more photons may arrive simultaneously at the XRF detector, limiting the number of photons the detector is able to count. By reducing the current used to produce the incident photons, a decreased photon emission from the sample would occur, which would decrease the amount of "dead time" of the measurement, thereby increasing the precision of the analysis. Alternatively, the use of new radiation detector technology capable of handling higher count rates, such as silicon drift detectors, would have a similar effect. Furthermore, if the voltage used to create the incident photons was increased, the energy spectrum resulting from irradiation of the sample would be shifted such that the backscatter spectra would produce less interference with the spectrum of interest (Roy et al., 2010).

Concordance correlation coefficient values tended to peak at the ≥ 20 mg mass categories in 7 of the 12 analyses (all nails, toenails only, fingernails only, for all data and <MDL truncated data, as well as for raw and scaled CCC results). These results (Table 5.6) indicated that a sample mass of at least 20 mg might be preferable to reach a high level of correlation between XRF and ICP-MS [As] values. The largest kappa value obtained was 0.636 at the 2.0 μg/g cut-off concentration for the fingernail samples that were at least 10 mg in mass, when ignoring those samples labelled as <MDL by ICP-MS (Table 5.8). Across all kappa analyses, there was a general trend toward increasing kappa values with increasing sample mass. While it was found that the scaled data was more highly and consistently

correlated between detection methods, it is important to recall that true correlations may be slightly lower than those scaled correlations observed here, and that the observed correlation values do not suggest very good correlation between tests.

Kappa values for [As] measurement between methods (Table 5.8) showed that while the 2.0 μg/g cut-off provided "substantial agreement" between ICP-MS and XRF, the 1 μg/g cut-off provided only "fair agreement", and the 1.4 μg/g cut-off "moderate agreement". This trend indicated that the [As] of a sample influenced the ability of XRF to agree with the ICP-MS, with higher concentration samples leading to greater agreement with ICP-MS. Additionally, the largest kappa values found at each cut-off concentration for all samples, toenails only and fingernails only was found to be in the <MDL truncated data set in 8 out of 14 analyses (57%), suggesting that the relatively high minimum detection limit of the XRF is a limiting factor in the agreement between [As] as measured by XRF and ICP-MS. Using the maximum contaminant limit of 1.0 μg/g of As as the concentration cut-off for identifying positive tests in all nail samples, the XRF had a kappa value of only 0.237 with respect to ICP-MS. This value is not high enough to suggest that XRF is, in its current form and function, equivalent to the ICP-MS.

In a clinical setting, the maximum contaminant limit set by the WHO is 1.0 μg/g of As in human nail tissue. At this cut-off concentration, the Sn and Sp of the XRF was 88.9% and 28.6% for all samples and 100.0% and 29.6% for fingernail samples, respectively (Table 5.10). At 1.0 μg/g, only 40.6% of fingernail samples

were correctly classified by XRF, representing substantial misclassification. While the XRF may not be suited for confirming suspected cases of As exposure in the field, it shows more potential for use as a screening tool to accurately detect negative samples, when used on fingernails especially. However, these figures are based on a small sample size (demonstrated by the wide 95% CIs) and should be interpreted with caution. There is not as of yet enough evidence to suggest that XRF is a suitable replacement for ICP-MS in a field setting.

For the linear regression analysis to determine which factors contributed to the difference between As measurements in nails between ICP-MS and XRF, each of the significant predictors corroborated the data analysis from the CCC and kappa analyses (Table 5.11). Fingernails were associated with a smaller difference between measurements (not significant), and an increasing sample mass also was associated with smaller differences. Previous research by Gherase and Fleming provided insight that the size (and therefore the mass) of nail clippings is associated with the XRF measurement of As in the sample (Gherase & Fleming, 2011). The mass attenuation coefficient, the amount of emitted x-rays that are absorbed by the sample and which do not simply pass through it, increases with the density and mass of a sample. A higher mass attenuation coefficient of a sample is associated with more emitted x-rays measured, and therefore the XRF will be able to more precisely quantify the amount of an element in a sample. Therefore, the finding that sample mass is associated with the difference between XRF and ICP-MS As measurements in nails was well-founded. These results provide more

evidence that the XRF may be best applied on samples with a high mass and best results may be found using fingernail samples.

As for CCC, kappa, and Sn and Sp values for As, results for agreement between testing methods of [Se] in nail samples was substantially higher in fingernails than in toenails. For raw and scaled analyses, the CCC value peaked in the >= 20 mg category in 5 of the 12 analyses, suggesting that a sample mass of at least 20 mg is preferable to reach high levels of agreement between the tests (Table 5.16). Additionally, the kappa (across all possible divisions and categories) increased with increasing [Se] cut-off, indicating that for the XRF to agree substantially with ICP-MS, a larger sample concentration would be beneficial (Table 5.18). In contrast to the As kappa analyses, there was no trend of increasing agreement between measurements with increasing sample mass, and there was evidence of decreasing agreement with increasing sample mass. It is not known why the performance of the XRF appears to be affected by mass more for As measurements than Se measurements, however it may be due to the fact that the concentration of Se in nail samples (as measured by ICP-MS) was higher than As (approximately 4.5 times higher, on average). Given a homogenous nail sample of size X, an identical nail sample of size 0.5*X will have half of the amount of As in it as the original X-sized sample. Therefore, in a given sample, a larger size and mass means a larger amount of As available in the sample to be detected by the XRF. Following this rationale, with the high MDL of the XRF, an increase in sample mass may be enough to increase the amount of As in the sample to a level which the XRF is able to more accurately measure, whereas the concentration of Se in a sample may already be

great enough that a similar increase in Se content due to a sample mass increase may not alter the XRF accuracy much. Regardless of the differences in values between nail types and masses, the kappa values observed for these analyses indicate that the XRF is not a suitable replacement for ICP-MS for determining the concentration of Se in nail samples.

With a Sn of 100% in fingernails at the 2.6 μg/g and 3.0 μg/g of Se cut-offs, the XRF was able to correctly identify each truly positive fingernail sample, without misclassification (Table 5.20). As well, with a NPV of 100%, if the XRF was used on fingernails in a field setting and sample tested negative, we could be quite confident that the sample truly was negative. While these values are high, the Sp of the XRF on fingernails is low, causing substantial misclassification. The finding that fingernails provide better Sn, Sp, PPV and NPV than toenail samples provides evidence to support the recommendation that fingernails be used as test samples in further test evaluations, as opposed to toenail samples.

The XRF showed higher correlation between nail As and Se concentrations than ICP-MS, which may be due to the "pre-selection" of higher quality As measurements by the XRF due to its higher detection limit for this element. The nearly linear increase between As and Se in nails is an interesting finding and has not been reported previously in published literature. Previous research has found that an increase in Se or As in the body (by ingestion or injection) can cause increased or decreased excretion of the other, depending on the organ/tissue investigated. For instance, an increase in As in the liver causes an increase in Se in

bile and the gastrointestinal tract (Gailer et al., 2002). Alternatively, increased ingested Se can cause decreases of As in hair, while increased As ingestion causes a decrease of Se in urine and lungs (volatile Se compounds are exhaled as a main excretion process of excess Se) (Levander, 1977; Levander & Baumann, 1966; Zeng, Uthus, & Combs, 2005). In the liver and blood of rabbits and other mammals, As-Se conjugates are formed (seleno-bis (S-glutathionyl) arsinium [$(GS)_2AsSe^-$] which are then released through the biliary duct to the gastrointestinal tract for excretion (Gailer et al., 2000, 2002; Gailer, 2007). With equal parts of As and Se in the $(GS)_2AsSe^-$ conjugate, it is possible that the nearly 1:1 linear increase of As and Se in nails could be accounted for by this conjugate. However, inorganic elements (As(III) and As(V)) are normally the major constituents in nails and it is not known if this As-Se conjugate, an organic compound, is deposited in nails, though small amounts of organic arsenicals can normally be detected (e.g. MMA^{5+} and DMA^{5+}) (Mandal, Ogra & Suzuki, 2003). If the As-Se conjugate is deposited in nails, variability in the linear relationship between As and Se in nails may be attributed to "left over" forms of either element that have not been formed into conjugates. Further studies into what influences the ratio of As:Se in human nails is warranted to explain these findings and to investigate this hypothesis. Since Se can be used as a therapeutic treatment for As poisoning (both acute and chronic), examining the relationship between these elements in nail simultaneously may be a major benefit of XRF analysis.

In linear regression analyses for the difference observed between XRF and ICP-MS results, for both As and Se, the concentration of the opposite element in nail

tissue was included in models as a potential confounding variable (i.e. for the As analysis, the concentration of Se in nails was investigated as a potential confounding variable, and vice versa). No confounding effects of As or Se in nail tissue were found in linear regression analyses, and therefore were not included in final models.

5.5 Conclusion

By performing concordance correlation coefficient and kappa analyses, determining Sn and Sp values, and performing linear regression analysis on the difference between XRF and ICP-MS As and Se concentration measurements in fingernail and toenails, a detailed description of the agreement between XRF results and ICP-MS results has been compiled.

It was found that, in most cases, an increasing sample mass was associated with better agreement between tests and negatively associated with the difference in measurements between tests. Fingernails were consistently found to have higher numerical (though not statistically significantly higher) agreement between tests as compared to toenail samples or all samples combined. The XRF was found to have an Sn and an NPV of 100% (Sp: 30%, PPV:21%) for fingernails at the 1.0 μg/g of As in nail cut-off concentration and an Sn and an NPV of 100% (Sp: 36%, PPV: 82%) for fingernails at a cut-off concentration of 2.6 μg/g of Se in nails. All Sn, Sp, PPV, and NPV were found to be higher for fingernail samples than for toenail samples or all samples combined.

It's lightweight and mobile construction make the XRF ideal for field use, and it is a more time- and cost-efficient test for As and Se in nail samples than ICP-MS. However, the present study has determined that the XRF is not currently a suitable replacement for ICP-MS analysis, with low overall correlation and agreement between the two tests. Future refinements, including adjustments in x-ray tube current and voltage settings, and tests of the XRF in field settings, which should be performed on fingernail samples rather than toenail samples, may prove to increase the usefulness of this novel diagnostic tool.

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6. Conclusion

6.1 Introduction

The overall goals of this thesis were to evaluate methods for the biomonitoring of arsenic (As) in people by: 1) assessing the accuracy of a novel test for As, 2: determining relationships between estimated ingestion exposures of As and biomarker, demographic and household factors; and 3) determining factors associated with [As] in a number of biological samples, including urine, hair, fingernails and toenails.

To this end, there were four specific objectives in this thesis: 1) to determine which factors and biological samples (urine, fingernails, toenails, and hair) were associated with estimated amount of As consumed via water, food, and a combination of both; 2) to determine which factors were associated with the concentration of As ([As]) in the urine, both total and speciated metabolites; 3) to determine which factors were associated with the total [As] in fingernails, toenails, and hair; and 4) to evaluate the accuracy of a novel method of quantifying [As] in human nail tissue via x-ray fluorescence (XRF). This chapter highlights the results for each of the above objectives, and provides an overall conclusion to the preceding research.

6.2 Understanding Self-reported Exposure to Arsenic of a Sample Population from Areas of Nova Scotia at Risk of Arsenic Exposure

The main objective of chapter two was to determine which demographic or lifestyle factors were associated with the estimated value of As consumption from

water, food and a combination of both. Secondly, this study aimed to determine which biological sample (fingernail, toenail, hair or urine) were associated with the estimate of past As exposure based on the collection of short-term exposure information. Results from this study help determine which biological samples are appropriate for sampling when attempting to use biological samples as surrogates of As exposure over a short time period, to eliminate the need to collect multiple samples.

Linear regression was used to investigate which biological samples, consumption habits, and demographic factors were most associated with estimates of how much As (natural log transformed to normalize the outcome variable) participants consumed from water in a 2-day period (median: 3.2 μg, range: 0 – 1,798.8 μg). As indicated in previous substantive chapters, in linear regression models with log-transformed outcomes, here and in other chapters, coefficients are on a multiplicative scale due to the transformation, and therefore, when back-transformed to the original scale, must be interpreted on a multiplicative scale, as a percent change when comparing two or more groups or levels. Therefore, significant associations from continuous independent variables will be interpreted as the percent change in the outcome variable that is associated with increasing the continuous independent variable value from the 25^{th} percentile to the 75^{th} percentile (i.e. the interquartile range). For categorical variables, all percent changes in the outcome variable will be interpreted with respect to the baseline category of the independent categorical variable.

Significant associated variables in the final multivariable model for As consumed from water were specific gravity (SG) adjusted total urinary [As], fingernail [As], water pH level, depth of the well used to draw drinking water, and the amount of As ingested from eating rice (Table 2.12). Participants with SG-adjusted total urinary [As] between 20 – 36 μg/L, 36 – 72 μg/L, and above 72 μg/L, were associated with 4.6 times (95% CI: 1.2 – 18.2), 8.8 times (95% CI: 2.1 – 37.0), and 8.9 times (95% CI: 2.1 – 37.7) more As consumed from water, respectively, compared to participants with < 9 μg/L SG-adjusted total urinary [As] ($p = 0.031$ overall). Participants whose primary source of home drinking water came from a well with a depth of between 42.1 – 55.0m had an associated increase in water As consumption of 5.4 times (95% CI: 1.4 – 21.5), in comparison to those whose well was less than 23.0 m deep. No other well depth categories predicted a significant increase or decrease in water As consumption ($p = 0.031$ overall), suggesting that rock and soil at this depth, at these locations, may have more As in water than in more shallow or deeper wells. An increase in fingernail [As] from the 25th to the 75th percentile (0.077 – 0.4 μg/g) was associated with an estimated 1.3-fold (95% CI: 1.1 – 1.6) increase in As consumed from water ($p = 0.010$), and a similar increase in the water pH level (25th percentile = 5.5; 75th percentile = 6.6) was associated with an increase of 6.1 times (95% CI: 3.2 – 11.6) the amount of As consumed from water ($p < 0.001$). At higher pH levels, As that is adsorbed to soils is more hydrologically available, and previous studies have shown that a high pH is associated with higher [As] in groundwater, particularly in reducing environments (Canadian Council of Ministers of the Environment, 1997; Kinniburgh & Smedley,

2002). Therefore, high water pH can contribute to increased water [As], which may lead to a higher consumption of As from water.

Two sets of logistic regression analysis were used to determine which factors were associated with high estimated As consumption from food, and from water and food combined (Table 2.13), because no transformations could make the two outcome variables normalized, making linear regression not possible. For the analysis of estimated As consumed from food, the median food As consumption value of 9.3 μg was used to dichotomize participants into exposed and unexposed groups. SG-adjusted total urinary [As] (categorized as above) and household income category were found to be significantly associated with high levels of As consumed from food. In this analysis, urine As categories 4 and 5 (36 – 72 μg/L and >72 μg/L, respectively) were found to differ significantly from the baseline (<9 μg/L). Having a urine sample in the 36 – 73 μg/L of As range increased a participant's odds of being in the exposed category (consuming at least 9.3 μg of arsenic from food in a 3-day period) by 8.0 times (95% CI: 2.3 – 27.6, p = 0.001), and a urine sample in the top 20% of [As] (>72 μg/L) had increased odds, by 9.4 times (95% CI: 2.8 – 31.8), of being in the exposed category (p < 0.001). Income categories were defined as: 1) <$30,000/year, 2) $30,000 - $50,000/year, and 3) >$50,000/year. With <$30,000/year as the baseline income level, participants earning between $30,000 - $50,000/year were 6.0 times (95% CI: 0.9 – 40.1) more likely to be in the exposed category (p = 0.064), and participants in the >$50,000/year income category were 6.6 times (95% CI: 1.2 – 36.6) more likely to

be in the exposed category (p = 0.031). This finding may largely be explained by the increase in seafood consumption in participants with increasing income category. The mean amount (with standard deviations, SD) of seafood consumption in different income categories were 0.7 oz. (SD 2.0 oz.), 2.9 oz. (SD 4.8 oz.), and 3.1 oz. (SD 5.5 oz.), for the <$30,000/year, $30,000-$50,000/year, and >$50,000/year categories, respectively.

In the logistic regression analysis for determining factors associated with estimated total As consumption from water and food combined (Table 2.13), the median consumption value of 86.4 μg was used to dichotomize participants into exposed and unexposed groups. SG-adjusted urinary [As] (categorized as above) and well depth (categorized in quintiles) were found to be significantly associated with the amount of total As consumed from water and food. Categories 4 and 5 (36 – 72 μg/L and >72 μg/L) of the SG-adjusted total urinary [As] were significantly different from the category 1 baseline level (<9 μg/L), increasing the odds of being in the exposed category by 5.8 times (95% CI: 1.4 – 23.9, p = 0.014) and 10.3 times (95% CI: 2.8 – 37.3, p < 0.001), respectively). As discovered for the prediction of water As consumption, the depth of the well used for a participant's main source of water (categorized into quintiles) was found to be associated with total As consumption. Wells with a depth of between 23.0 – 41.0m were found to be associated with an increase in the odds of being in the exposed category by 8.8 times (95% CI: 1.9 – 42.1), as compared to participants whose wells were <23 m deep. Previous research has found that the [As] in the earth's crust increased with increasing depth up to 22 m, and then decreased afterward (Chowdhury et al.,

1999), though this research was performed in the Ganges Delta of Bangladesh and West Bengal, India. Others have reported [As] both increasing (Becker, Smith, Greer, et al., 2010; Kim, Miranda, Tootoo, et al., 2011; Kim, Nriagu & Haack, 2003; McCurdy, 1980) and decreasing with well depth (Kim et al., 2003), and it is likely that the association between well depth and water [As] is highly dependent upon the geology of individual locations, suggesting that surveys of soil and rock should be performed before wells used for drinking water are installed.

In each regression analysis, SG-adjusted total urinary [As] was found to be a significantly associated with the outcome, and was the only variable to appear across all regression models. Only one other tested biomarker of arsenic exposure, fingernails, was significantly associated with the outcomes, and only in one analysis (water As consumption, Table 2.12). These results demonstrate that, as surrogates of As consumption over short-term exposure (2-day consumption for water, 3-day consumption for food), urinary [As], adjusted by specific gravity (or possibly other urine constituents such as creatinine), is a good biomarker. While fingernail [As] may be a suitable alternative biological sample as a surrogate of water As consumption, toenail and hair samples were not found to be useful in predicting As exposure, from any source, using short-term exposure data. Our results also indicate that knowledge of the geographically distinct range and depth of arsenic-rich soil may be able to be used to prevent new wells being drilled into areas at high-risk of arsenic contamination.

6.3 Factors Associated with Urine Arsenic Metabolites

The main goal of the third chapter was to investigate which demographic and lifestyle factors predicted the concentration of each of several urinary As metabolites – total arsenic, arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA(V)), dimethylarsinic acid (DMA(V)), arsenobetaine (AsB) and total arsenic minus arsenobetaine (TMA).

Multivariable linear regression analyses were run for each of the SG-adjusted urinary As metabolites (natural log transformed to normalize the outcome variable). Logistic regression analyses were run for SG-adjusted urinary As(III), As(V), and MMA(V), due to low proportions of participants with urine samples above the MDL for these metabolites.

The most consistent positive linear regression predictor across all urinary metabolites was water [As](Table 3.9); an increase in water [As] was associated with increases of As in the form of As(III), As(V), MMA(V), and DMA(V). Only in the urinary AsB regression analysis was water [As] not a significant predictor, as expected. Participants over 50 years of age were predicted to have a total urinary [As] of 1.7-fold (95% CI: 1.2 – 2.3) higher than younger participants, and an increase in age from the 25th to the 75th percentile (47 to 68 years) was associated with an increase of 1.6-fold (95% CI: 1.2 – 2.2) more urinary AsB. These associations may be partially explained by the observation that seafood consumption increased with age (mean consumption values of 47.3 g (95% CI 2.4 – 92.1 g), 68.2 g (95% CI 39.0 – 97.4 g), and 101.2 g (95% CI 64.9 – 137.4 g) were

observed for participants aged <40, 40-60, and >60, respectively), likely contributing substantially in higher [AsB] and total [As] in urine. A positive relationship existed between water [As] and predicted urinary MMA(V) and DMA(V) concentrations, but this relationship was stronger in Hubbards than in Fall River, with a significant interaction between location and water [As] found in both regression analyses (Figures 3.1 and 3.2). In linear regression analyses, participants from Fall River were predicted to have a urinary [MMA(V)] 57% of those from Hubbards – a small, but significant difference ($p = 0.007$) – in the highest water [As] category. Similarly, participants from Fall River were predicted to have a urinary [DMA(V)] 55% of those from Hubbards – a slightly larger, even more significant difference ($p = 0.003$) – in the highest water [As] category. Fall River is known to have elevated levels of As in soil, with its history of gold mining, whereas Hubbards does not. With potential sources of As exposure unaccounted for in this study – soil and air – the relationship between water [As] and specific urinary As metabolites may appear to be stronger in Hubbards, where a more complete picture of participant's exposures is known.

Seafood consumption was a significant predictor of total urinary As, As(III), DMA(V), AsB, and TMA concentrations (Table 3.9). The finding that ingested seafood was positively associated with urinary [DMA(V)]; an increase in seafood consumption from the 25th to the 75th percentile (0 g to 4 oz. or 113.2 g) predicted an increase in urinary [DMA(V)] of approximately 1.4 times (95% CI: 1.3 – 1.5). This supports evidence that arsenosugars, arsenolipids, or arsenocholine can be metabolized to DMA(V) in humans (Francesconi, Tanggaar, McKenzie, et al., 2002;

Navas-Acien, Francesconi, Silbergeld, et al., 2011), while AsB is excreted unchanged in the urine. The positive association between seafood consumption and urinary [As(III)] suggests that seafood-derived As compounds may potentially be metabolized in the body to highly toxic forms of inorganic As, such as As(III). This is a novel finding and more research is required to substantiate this hypothesis.

Contradictory findings surrounding consumption of chicken were found for linear and logistic regression analyses for urinary [As(V)]. Linear regression (Table 3.9) predicted a decrease in urinary [As(V)], while logistic regression analysis (Table 3.11) predicted an increase in urinary [As(V)], with reported chicken consumption in the 3 days prior to urine collection. While previous studies have found that chicken consumption can lead to significant increases is total As consumption as well as positive associations As in human biological samples (Lasky et al., 2004; Rivera-Nunez et al., 2011), the results of the present study are conflicting. However, with the low number of observations in the linear regression analysis for urinary As(V) (n=52), the results of the logistic regression analysis (n=166) are likely to be more representative of the true association between chicken consumption and urinary [As(V)].

The pH level of a participant's main water source was a significant positive predictor of MMA(V) (linear regression only, Table 3.9), DMA(V) and TMA concentrations, increasing these urinary metabolites by 1.3-fold (95% CI: 1.1 – 1.6), 1.3-fold (95% CI: 1.1 -1.5), and 1.4-fold (95% CI: 1.2 – 1.7), respectively, for an increase in water pH from the 25th to the 75th percentile (5.5 to 6.6). As mentioned

in the previous section, at higher pH levels, As that is adsorbed to soils is more hydrologically available. Therefore, high water pH can contribute to increased accumulation in water, which subsequently increases the concentration of urinary TMA, MMA(V), and DMA(V).

Each urinary As metabolite has different health outcomes associated with exposure, over both long- and short-term periods (Kitchin, 2001; Kligerman et al., 2003; Mass et al., 2001; Thomas, Styblo & Lin, 2001). For example, inorganic As species are more acutely toxic than organic As species, which cause longer-term genetic damage. Our research suggests that the concentration of urinary As metabolites can come from many routes of possible exposure, and these routes should be considered when using urinary As metabolites in As biomonitoring programs to minimize the health effects of As exposure. In addition, if urinary [As] is being used as a biological marker of exposure, as was found to be appropriate in Chapter 2, many factors may influence whether or not total urinary As, or any urinary As metabolite, is found to be a significant predictor of exposure. Finally, the metabolism of As compounds derived from seafood should be investigated further to determine if their metabolism might lead to the formation of potentially dangerous As metabolites in humans.

6.4 Factors Associated with Arsenic Concentrations in Hair, Fingernails, and Toenails

The objective of the fourth chapter was to investigate which factors were associated with the concentration of total As found in hair, fingernails, and toenail

samples. The factors that were related to the [As] in all three biological samples were remarkably similar. In each case, water [As] was highly positively predictive of the concentration of As within each sample. From logistic regression analyses, the odds of a hair sample containing more As than the MDL (0.068 μg/g) increased by 2.7 times (95% CI: 1.1 – 7.0) and 7.4 times (95% CI: 2.2 – 25.6, $p < 0.001$) for participants whose water contained between 20 – 50 μg/L or > 50 μg/L, respectively, compared to those with less than 10 μg/L (Table 4.3). For fingernails, the odds of a sample having more As than the MDL (0.153 μg/g) significantly increased, by 4.4-fold (95% CI: 1.9 – 10.3) for people whose water contained more than 10 μg/L of As (Table 4.9). The odds of a toenail sample having more than the MDL (0.153 μg/g) of As increased by 6.8-fold (95% CI: 2.7 – 17.0) for participants whose water had > 10 μg/L of As compared to those with < 10 μg/L (Table 4.9).

As well, the concentration of selenium (Se) in each sample was highly positively correlated with [As], after controlling for Se consumption from water. In linear regression analyses, hair [Se] was positively associated with hair [As], with a predicted increase of 1.03 times (95% CI: 1.01 – 1.05) the concentration of As in hair for an increase in hair [Se] from the 25th to the 75th percentile (0.2 – 0.7 μg/g) (Table 4.5). From logistic regression results, the odds of having a hair [As] of greater than the MDL increased by 3.0 times (95% CI: 1.2 – 7.8) and 5.7 times (95% CI: 2.1 – 15.8) for participants with hair [Se] of between 0.23 – 0.43 μg/g and > 0.42 μg/g, respectively, compared to those with < 0.23 μg/g (Table 4.3). Fingernail [As] was predicted to increase by 2.8-fold (95% CI: 1.8 – 4.1) for participants with a nail

[Se] of > 2.6 μg/g compared to those with a nail [Se] < 0.7 μg/g (Table 4.12). As well, the odds of having a fingernail [As] of greater than the MDL increased by 4.6-fold (95% CI: 1.4 – 15.1) and 11.6-fold (95% CI: 3.2 – 41.8), for participants with fingernail [Se] of between 0.9 – 2.3 μg/g, and >2.3 μg/g, respectively, compared to those with less than 0.6 μg/g (Table 4.9). An increase of 2.7 times (95% CI: 1.78 – 4.1) the amount of As was predicted in toenails for samples with a toenail [Se] of more than 2.1 μg/g compared to those with less than 0.7 μg/g (Table 4.12). The odds of a toenail sample having more than the MDL of As increased by 15.1 times (95% CI: 3.5 – 65.5, $p < 0.001$) for participants with a toenail [Se] of > 1.5 μg/g, compared to those with a toenail [Se] < 0.6 μg/g (Table 4.9). Additionally, the amount of seafood consumed was a significant positive predictor in the logistic regression analysis of dichotomized fingernail [As], categorized as <MDL or ≥MDL, accounting for an increase in odds of being in the high fingernail [As] category by 4.5 times (95% CI: 4.1 – 4.9) for participants who consumed 4 oz. of seafood (113.2 g) compared to those who consumed none (equal to the interquartile range).

The relationship found between As and Se in hair, fingernails, and toenails provides further suggestion that nail or hair studies related to As consumption or exposure should also consider Se in their analyses. The metabolism of As and Se are closely related, with each element causing increased excretion (via exhalation, urination, or the gastrointestinal tract) of the other in a mutual detoxification (Biswas, Talukder & Sharma, 1999; Gailer, 2007; Wang, Yang, Hou, et al., 2001). The arsenic-selenium conjugate compound seleno-bis (S-glutathionyl) arsinium ion

$[(GS)_2AsSe]^-$ is a major metabolite in the processing of As and Se. While the $[(GS)_2AsSe]^-$ metabolite is present in blood and digestive secretions (Gailer et al., 2002), it is not known if it plays a role in the excretion of As and Se through solid tissues such as hair and nails. Selenium can be an effective tool in decreasing the toxic effects of As exposure (Wang et al., 2001). For instance, people consuming high levels of As through drinking water can show marked decreases in tissue [As] after supplementation with Se (Zeng, Uthus & Combs, 2005). With As and Se concentrations being so closely related in hair and nail tissue, further research would be useful to determine the constituents of these elements in tissues and how the ratio between [As] and [Se] may predict the potential health outcomes of arsenic toxicity.

6.5 Evaluation of a Novel Method of Arsenic Detection in Human Nail Samples by X-ray Fluorescence

The main goal of the fifth chapter was to evaluate a novel method of quantifying As and Se in human nail samples via x-ray fluorescence (XRF), as compared to inductively coupled plasma-mass spectrometry (ICP-MS).

To compare the two detection methods, concordance correlation coefficient (CCC), kappa, sensitivity (Sn) and specificity (Sp), and multivariable linear regression analyses were performed. Concordance correlation coefficient analyses were performed on raw data as well as on data that had been scaled linearly, using a reduced major axis analysis. The scaled data were more representative of a 1:1

relationship between the XRF and ICP-MS measurements; the goal of scaling the data was to more accurately represent the true relationship between the measurement methods. However, while the raw data may under-represent the true correlation between measurement methods, since the scaled data are scaled based upon the *existing* data, they may over-represent the correlation between the two methods.

Concordance correlation coefficient results showed that fingernail samples most consistently provided the highest level of correlation between methods, and especially when samples were at least 20 mg, though overall the XRF showed low coefficient values (Table 5.6). The largest scaled CCC for As analyses, 0.560 (95% CI: 0.172 – 0.948), was found for fingernail samples of at least 20 mg. Kappa analyses found that the highest agreement, beyond that due to chance alone, between XRF and ICP-MS at a clinically relevant cut-off concentration of 1.0 μg/g of As in nail was observed for fingernail samples of at least 30 mg (k = 0.237, 95% CI: -0.068 – 0.543) (Table 5.8). Higher kappa values were observed at higher cut-off concentrations of 1.4 μg/g and 2.0 μg/g (k = 0.397 and k = 0.327, respectively), suggesting that the XRF was more accurate at higher [As] in these nail samples, but kappa values overall were not very high.

Sn and Sp analyses found that, at a cut-off concentration of 1.0 μg/g of As in nail, fingernail samples were again superior to toenails in terms of the XRFs ability to correctly identify a positive or negative sample. For fingernails at the 1.0 μg/g cut-off concentration, the XRF had a Sn of 100% (95% CI: 48 – 100%) and an NPV

of 100% (95% CI: 63 – 100%), greatly improving on the toenail results, as well as for all nails combined. However, at this cut-off, the XRF had a Sp = 30% (95% CI: 14 – 50%) and PPV = 21% (95% CI: 7 – 42%), leading to substantial numbers of samples being falsely categorized as above 1.0 μg/g by the XRF compared to the ICP-MS (Table 5.10).

Multivariable linear regression analyses found that the difference in measurements between XRF and ICP-MS, for all nail samples, was negatively associated with sample mass (non-linear, quadratic relationship) and fingernails (Table 5.11). The difference between XRF and ICP-MS decreased with increasing sample mass, declining from greater than 10 μg/g to 0 μg/g after sample mass increased from 0.3 mg to ~30 mg. Toenails were found to have, on average, a 1.2 times larger difference (95% CI: 0.6 – 2.1, p = 0.660) between XRF and ICP-MS measurements than fingernails.

To compare the methods of Se quantification, the same analyses were performed as for As analyses. Results showed once again that fingernail samples most consistently provided the highest level of correlation between methods of Se detection, and especially when samples were at least 20 mg, although overall correlation between detection methods was again low. In general, CCC values were higher for the Se data than they were for As data, with a maximum scaled value of 0.548 (95% CI: 0.331 – 0.765) found for all available fingernail samples (Table 5.16). Kappa values for the agreement, beyond that due to chance, between XRF and ICP-MS analysis of [Se], increased with increasing cut-off concentrations: k =

0.354 (95% CI: 0.030 – 0.677), k = 0.429 (95% CI: 0.063 – 0.796), and k = 0.484 (95% CI: 0.105 – 0.864), for the 2.0 μg/g, 2.6 μg/g, 3.0 μg/g cut-off values, respectively (Table 5.18). Fingernails consistently provided higher kappa values than toenails. Sn and Sp analyses found that, at a cut-off concentration of 2.0 μg/g of Se in nail, fingernail samples were again superior to toenails in terms of the XRFs ability to correctly identify a positive or negative sample. For fingernails, at the 2.6 μg/g and 3.0 μg/g cut-off concentrations, the XRF had a Sn of 100% (95% CI: 89 – 100%) and an NPV of 100% (95% CI: 40 – 100%), but had a Sp of 36% (95% CI: 11 – 69%) and a PPV of 82% (95% CI: 66 – 92%), better than the toenail results, as well as for all nails combined, but still showing large numbers of misclassified samples (Table 5.20).

Overall, we found that the XRF is not currently well-suited as a replacement for ICP-MS. High Sn and NPV values suggest that, if the XRF were used in the field, it would be best suited as a screening tool in high-risk areas. Because of a very low Sp and PPV, especially for As analyses, any sample that tested positive would need confirmation using a more specific method, such as ICP-MS. More calibration and research is needed before the XRF can function as a replacement for more sensitive, specific, and accurate methods of As detection, such as ICP-MS.

6.6 Overall Conclusions and Recommendations

Future studies and programs using biological markers of As exposure should focus efforts on collecting urine samples when attempting to assess short-term exposure to As. As well, any further research involving urine as a biological marker

of exposure to As should take specific urinary As metabolites into consideration and pay special attention to seafood consumption, which can strongly affect urinary [As], and can potentially lead to an increase in toxic inorganic As metabolites. When urine is not available or desired for such studies, fingernails are the preferred biological tissue to be used as a surrogate of As exposure, rather than toenails or hair. Studies focusing on As in hair or nails should also consider the total and relative concentrations of Se in respective samples, as they appear to be highly correlated and can potentially be used to predict adverse health outcomes.

Additional research on the novel XRF method of As quantification is warranted to increase its Sp and PPV before it can be considered as a suitable replacement method for ICP-MS. However, with its high Sn and NPV (relative to Sp and PPV), further work may be well advised to focus on screening high-risk populations that are suspected of being exposed to As, while confirmatory analysis should be left to more specific devices. Any further studies using the XRF method of As detection should focus on fingernails, rather than toenails, of at least 20 mg, as these consistently provided more accurate [As] measurements than samples less than 20mg.

While As biomonitoring studies continue to use urine, hair, and nails, as surrogates of As exposure, further investigation into how appropriate each of these biological samples are, and for which timeline of As exposure, should continue. While this study has suggested that hair and toenails may not be as highly

associated with short-term estimated As consumption as commonly held, it is unclear as to how this relationship holds over longer times periods and under different circumstances. Studies involving more participants, and a longer follow-up period, would be beneficial to further elucidate these associations. Furthermore, studies should be conducted to determine what effect the depth of a well has on the concentration of As in the water found at these depths, and how this association varies geographically. This study has found that wells between 23 – 41 m were associated with higher estimated amounts of As consumed by participants, by way of higher [As] in water. This study has only examined two locations, by non-random sampling, and cannot be extrapolated to other areas. Geographically representative information about the relationship between soil/well depth and water [As] could be a valuable asset to persons intending to dig new wells in the future.

In the area of urine As biomonitoring, significant advances remain to be achieved in the area of speciation, and specifically in the speciation of As molecules and compounds stemming from the consumption of seafood. While much work has been done, further elucidation of chemical structure and toxicity of the plethora of seafood-derived As metabolites is warranted to help understand the impact of As consumption through seafood. While some seafood-derived As metabolites are believed to be innocuous, their metabolic pathways are yet to be determined, and therefore their true effects on humans cannot be known with certainty.

While the XRF system evaluated in this study has shown some limited promise, further refinements and studies are required. As mentioned in chapter 5, several steps could be taken to increase the precision of the XRF device. Studies should be undertaken which evaluate various methods of XRF refinement, including x-ray tube current reduction, improved radiation detection technology, increased voltage in creating incident photons. Future trials of the XRF system should be focused on fingernails of at least 20 mg in mass, as shown by our results, and should be evaluated on several different populations, with both high and low expected As exposures, to further evaluate the XRFs ideal area of usage.

6.7 References

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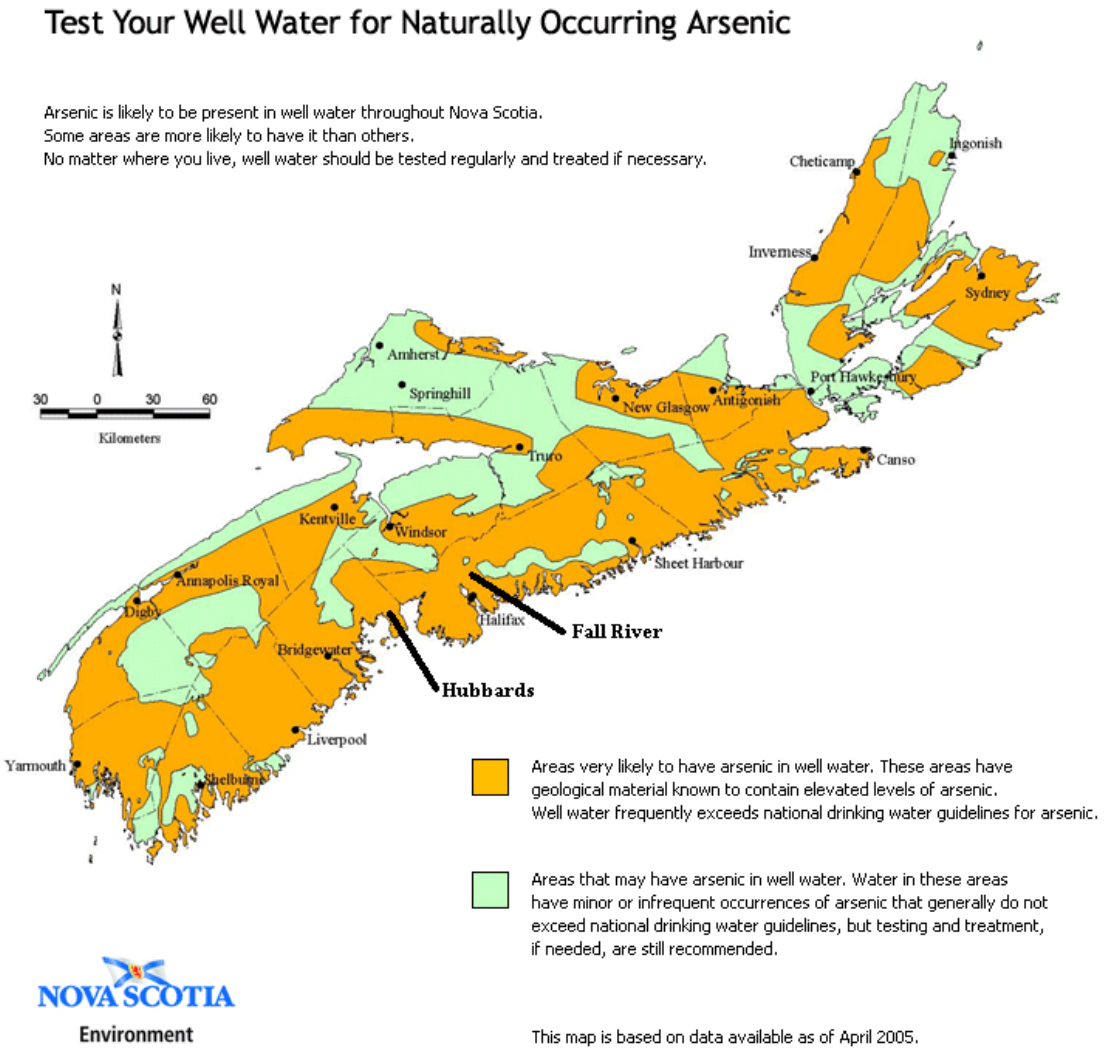
APPENDICIES

APPENDIX A

Map of Nova Scotia, Canada, indicating areas that may be at high risk of having high levels of arsenic in well water.

Obtained from Nova Scotia Environment, October 14, 2012, http://www.gov.ns.ca/nse/water/waterquality.natural.water.contaminants.asp



APPENDIX B

Participant questionnaire

FOOD AND DRINKING HABITS INTERVIEW SCRIPT

GPS LOCATION: _____
PARTICIPANT NUMBER:_____
SURVEY DATE:_____
INTERVIEWER:_____

RELEVANT FOOD AND WATER CONSUMPTION QUESTIONS:

1A) HOW MANY GLASSES OF WATER DID YOU DRINK YESTERDAY OF YOUR HOME TAP WATER.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

1B) HOW MANY GLASSES OF WATER DID YOU DRINK THE DAY BEFORE YESTERDAY OF YOUR HOME TAP WATER.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

2A) HOW MANY CUPS OF COFFEE MADE WITH YOUR HOME TAP WATER DID YOU DRINK YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

2B) HOW MANY CUPS OF COFFEE MADE WITH YOUR HOME TAP WATER DID YOU DRINK THE DAY BEFORE YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

3A) HOW MANY CUPS OF TEA MADE WITH YOUR HOME TAP WATER DID YOU DRINK YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

3B) HOW MANY CUPS OF TEA MADE WITH YOUR HOME TAP WATER DRUNK THE DAY BEFORE YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

4A) HOW MANY CUPS OF OTHER HOT DRINKS (E.G., HOT CHOCOLATE, ETC) MADE WITH YOUR HOME TAP WATER DID YOU DRINK YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

4B) HOW MANY CUPS OF OTHER HOT DRINKS (E.G., HOT CHOCOLATE, ETC) MADE WITH YOUR HOME TAP WATER DID YOU DRINK THE DAY BEFORE YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

5A) HOW MANY CUPS OF COLD DRINKS (SUCH AS JUICES, ICED TEA, LEMONADE, TANG, ETC) MADE WITH YOUR HOME TAP WATER DID YOU DRINK YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

5B) HOW MANY CUPS OF COLD DRINKS (SUCH AS JUICES, ICED TEA, LEMONADE, TANG, ETC) MADE WITH YOUR HOME TAP WATER DID YOU DRINK THE DAY BEFORE YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

6A) HOW MANY BEER DID YOU DRINK IN THE PAST 24 HOURS?
(1 BEER = 12 OZ BOTTLE OR CAN)
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 15]

6B) IF YES, WAS THE BEER HOME MADE WITH HOME TAP WATER?
1. YES
2. NO

6C) HOW MANY GLASSES OF WINE DID YOU DRINK IN THE PAST 24 HOURS?
(1 GLASS OF WINE = 4 OZ)
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 15]

6D) IF YES, WAS THE WINE HOME MADE WITH HOME TAP WATER?
1. YES
2. NO

6E) HOW MANY DRINKS WITH LIQUOR DID YOU DRINK IN THE PAST 24 HOURS?
(1 DRINK = 1 OZ OF LIQUOR)
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 15]

6F) IF YES, WAS THE LIQUOR HOME MADE WITH HOME TAP WATER?
1. YES
2. NO

7A) DID YOU EAT RICE OR RICE BREAKFAST CEREALS IN THE LAST 3 DAYS?
1. YES
2. NO
99. NOT ASCERTAINED

7B) IF YES, WHAT, WHEN AND HOW MUCH? PLEASE PROVIDE AS MUCH DETAILS AS POSSIBLE FOR EACH TIME THAT YOU ATE RICE OR RICE CEREALS.

_____
_____

8A) DID YOU EAT SEAFOOD OR FRESHWATER FISH IN THE LAST 3 DAYS?
1. YES
2. NO
99. NOT ASCERTAINED

8B) IF YES, WHAT, WHEN AND HOW MUCH? PLEASE PROVIDE AS MUCH DETAIL AS POSSIBLE FOR EACH TIME THAT YOU ATE FRESHWATER FISH OR SEAFOOD

_____
_____
_____

9A) DID YOU EAT CHICKEN IN THE LAST 3 DAYS?
1. YES
2. NO
99. NOT ASCERTAINED

9B) IF YES, WHAT, WHEN AND HOW MUCH? PLEASE PROVIDE AS MUCH DETAILS AS POSSIBLE FOR EACH TIME THAT YOU ATE CHICKEN.

_____
_____
_____

10A) HOW MANY CUPS OF OATMEAL OR OTHER COOKED CEREAL MADE WITH HOME TAP WATER DID YOU EAT YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

10B) HOW MANY CUPS OF OATMEAL OR OTHER COOKED CEREAL MADE WITH HOME TAP WATER DID YOU EAT THE DAY BEFORE YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

11A) HOW MANY CUPS OF COUS-COUS OR OTHER COOKED GRAINS MADE WITH HOME TAP WATER DID YOU EAT YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

11B) HOW MANY CUPS OF COUS-COUS OR OTHER COOKED GRAINS MADE WITH HOME TAP WATER DID YOU EAT THE DAY BEFORE YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

12A) HOW MANY CUPS OF PASTA MADE WITH HOME TAP WATER DID YOU EAT YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

12B) HOW MANY CUPS OF PASTA MADE WITH HOME TAP WATER DID YOU EAT THE DAY BEFORE YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

13A) HOW MANY CUPS OF SOUP MADE WITH HOME TAP WATER DID YOU EAT YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

13B) HOW MANY CUPS OF SOUP MADE WITH HOME TAP WATER DID YOU EAT THE DAY BEFORE YESTERDAY.
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

14A) HOW MANY CUPS OF OTHER FOODS MADE WITH HOME TAP WATER DID YOU EAT YESTERDAY. (PLEASE INDICATE THE TYPE OF FOOD_____)
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

14B) HOW MANY CUPS OF OTHER FOODS MADE WITH HOME TAP WATER DID YOU EAT THE DAY BEFORE YESTERDAY. (PLEASE INDICATE THE FOOD_____)
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

15A) DO YOU HAVE YOUR OWN VEGETABLE GARDEN?
1. YES
2. NO
99. NOT ASCERTAINED

15B) IF YOU HAVE A VEGETABLE GARDEN, HOW MANY CUPS OF VEGETABLES YOU HAVE GROWN DID YOU EAT YESTERDAY (FRESH, FROZEN OR PRESERVED)?
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

15C) IF YOU HAVE A VEGETABLE GARDEN, HOW MANY CUPS OF VEGETABLES YOU HAVE GROWN DID YOU EAT THE DAY BEFORE YESTERDAY (EITHER FRESH, FROZEN OR PRESERVED).
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

15D) PLEASE LIST THE VEGETABLES YOU GROW IN YOUR GARDEN
_____
_____
_____

16A) DO YOU GROW FRUIT ON YOUR PROPERTY?
1. YES
2. NO
99. NOT ASCERTAINED

16B) IF YOU GROW FRUIT, HOW MANY CUPS OF FRUIT YOU HAVE GROWN DID YOU EAT YESTERDAY (EITHER FRESH, FROZEN OR PRESERVED).
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

16C) IF YOU GROW FRUIT, HOW MANY CUPS OF FRUIT YOU HAVE GROWN DID YOU EAT THE DAY BEFORE YESTERDAY (EITHER FRESH, FROZEN OR PRESERVED).
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

16D) PLEASE LIST THE FRUIT YOU GROW

_____
_____
_____

17A) IN THE PAST 24 HOURS, HAVE YOU TAKEN ANY ANTACIDS OR CALCIUM-SUPPLEMENTS THAT WERE NOT PART OF A DAILY MULTI-VITAMIN?
1. YES
2. NO
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

17B) IF YES, PLEASE PROVIDE THE BRAND NAME OF ANTACID OR CALCIUM-SUPPLEMENTS TAKEN IN PAST 24 HOURS.
[_____]

18) IN THE PAST 10 DAYS, DID YOU TAKE ANY ORAL ANTIBIOTICS?
1. YES
2. NO
99. NOT ASCERTAINED

19A) ARE YOU CURRENTLY USING ANY OVER-THE-COUNTER OR PRESCRIPTION MEDICATIONS OR VITAMIN SUPPLEMENTS THAT ARE TAKEN AT LEAST ONCE PER DAY? (PLEASE DO NOT INCLUDE ANTACIDS, CALCIUM SUPPLEMENTS, OR ANTIBIOTICS)
1. YES
2. NO
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

19B) IF YES, PLEASE SPECIFY WHICH
|_____|

20A) IN THE PAST 24 HOURS, WERE YOU EXPOSED TO CIGARETTE SMOKE?
1. YES
2. NO
99. NOT ASCERTAINED

20B) IF YES, PLEASE DESCRIBE HOW MANY TIMES, WHERE AND FOR HOW LONG (FOR EACH TIME)? PLEASE PROVIDE AS MUCH DETAILS AS POSSIBLE FOR EACH TIME.
_____
_____
_____

21) HOW OFTEN DO YOU USE HOME TAP WATER FOR BATHING OR SHOWERING PER WEEK?
99. NOT ASCERTAINED
|_____| [RANGE: 0 TO 20]

22) DO YOU USE HOME TAP WATER FOR LAUNDRY?
1. YES
2. NO
99. NOT ASCERTAINED

23) DO YOU TREAT YOUR HOME WATER WITH A WATER SOFTENER?
1. YES
2. NO
99. NOT ASCERTAINED

24A) DO YOU USE ANY OTHER WATER TREATMENT EQUIPMENT?
1. YES
2. NO
99. NOT ASCERTAINED

24B) IF YOU HAVE OTHER WATER TREATMENT EQUIPMENT, PLEASE DESCRIBE THE TYPE, WHAT IT IS USED FOR (E.G., JUST FOR WATER YOU DRINK) AND WHEN YOU STARTED USING THIS EQUIPMENT.

_____
_____
_____

25) IN THE LAST WEEK DID YOU USE A SHAMPOO THAT CONTAINS SELENIUM (I.E., ANTI-DANDRUFF SHAMPOO)? YOU MAY WANT TO GO TO THE SHAMPOO CONTAINER AND LOOK AT ITS INGREDIENTS.
1. YES
2. NO
8. UNKNOWN
99. NOT ASCERTAINED

26) DO YOU DYE YOUR HAIR?
1. YES
2. NO
99. NOT ASCERTAINED

26B) IF YES, WHEN DID YOU LAST DYE YOUR HAIR? _____

27) IF YOU HAVE A PRIVATE WELL, HOW DEEP IS THIS WELL?
(9999 = NOT ASCERTAINED)
_____ (0 TO 400FT)

DEMOGRAPHIC PROFILE QUESTIONS:

28) PEOPLE LIVING IN CANADA COME FROM MANY DIFFERENT CULTURAL AND RACIAL BACKGROUNDS. ARE YOU -- (INTERVIEWER: Read categories to respondent. Mark all that apply.)

CATEGORY	**PERCENT**
1. WHITE	
2. BLACK	
3. ABORIGINAL PEOPLES OF NORTH AMERICA (NORTH AMERICAN INDIAN, MÉTIS, INUIT / ESKIMO)	
4. CHINESE	
5. JAPANESE	
6. KOREAN	
7. SOUTH ASIAN (E.G., EAST INDIAN, PAKISTANI, SRI LANKAN, ETC)	
8. SOUTHEAST ASIAN (E.G., CAMBODIAN, INDONESIAN, LAOTIAN, VIETNAMESE, ETC.)	
9. FILIPINO	
10. LATIN AND CENTRAL AMERICAN	
11. ARAB	
12. WEST ASIAN (E.G., AFGHAN, IRANIAN, ETC.)	
OTHER (SPECIFY:_____)	

99. NOT ASCERTAINED

29) AGE
1. 25 – 49 YEARS
2. 50 – 75 YEARS
99. NOT ASCERTAINED

30) GENDER
1. MALE
2. FEMALE
99. NOT ASCERTAINED

31) THE HIGHEST LEVEL OF EDUCATION OBTAINED
1. PRIMARY
2. SECONDARY
3. COLLEGE

4. UNIVERSITY
99. NOT ASCERTAINED

32) CURRENT OCCUPATION IN A PRIMARY INDUSTRY*
1. YES
2. NO
99. NOT ASCERTAINED
*PRIMARY INDUSTRY INCLUDES FARMING, FISHING, MINING (WORKING ON THE LAND OR SEA)

33) COMBINED HOUSEHOLD INCOME CATEGORY
1. <$30,000/YR
2. $30,000-$50, 000/YR
3. >$50,000/YR
99. NOT ASCERTAINED

34) FAMILY OWNS THE HOUSE IN WHICH THEY LIVE
1. YES
2. NO
99. NOT ASCERTAINED

35) CONSECUTIVE LENGTH OF TIME LIVED AT PRESENT HOME
1. 3 to 5 YEARS
2. 5 to 10 YEARS
3. 10 to 25 YEARS
4. >25 YEARS
99. NOT ASCERTAINED

36) CONSECUTIVE LENGTH OF TIME LIVED IN THE COMMUNITY
1. 3 to 5 YEARS
2. 5 to 10 YEARS
3. 10 to 25 YEARS
4. >25 YEARS
99. NOT ASCERTAINED

SAMPLE COLLECTION QUESTIONS:

37) TIME AND DATE WHEN URINE SAMPLE WAS COLLECTED
TIME: _____ AM/PM DATE: _____

38) TIME AND DATE WHEN TOENAIL SAMPLE WAS COLLECTED
TIME: _____ AM/PM DATE: _____

39) TIME AND DATE WHEN HAIR SAMPLE WAS COLLECTED
TIME: _____ AM/PM DATE: _____

40) TIME AND DATE WHEN CHEEK SWAB SAMPLE WAS COLLECTED
TIME: _____ AM/PM DATE: _____

41) TIME AND DATE WHEN WELL WATER SAMPLE WAS COLLECTED (PRE AND POST WATER SOFTENER OR TREATMENT, IF APPLICABLE)
TIME: _____ AM/PM DATE: _____

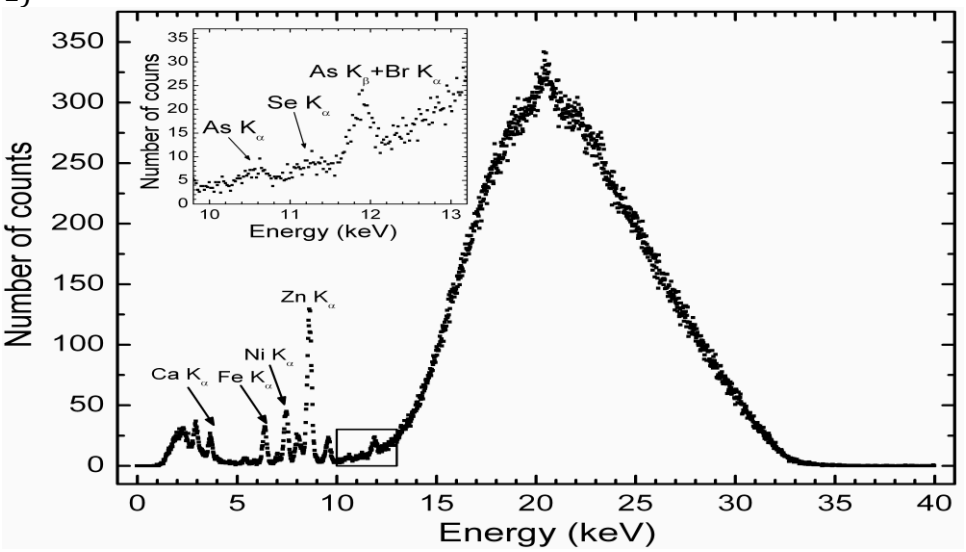
MAILING ADDRESS FOR SENDING CHEQUE AND LABORATORY RESULTS:
_____
_____
_____
_____
_____

E-MAIL ADDRESS:
_____

APPENDIX C

1) A typical energy spectra obtained from x-ray fluorescence of phantom nail samples containing arsenic and selenium.
2) a) The averaged measured spectrum (number of energy counts per second) of five repeated measurements of a single sample, by XRF, and the mathematically fit line of best fit; b) A single spectrum measurement (number of energy counts per second) from a single sample, showing more variation than in the average spectrum of five measurements.

1)



2)